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Factors Affecting Apple Hardiness and Methods of Measuring Resistance of Tissue to Low Temperature Injury

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ON THE COVER

The tree in the cover illustration shows the combination of hardy intermediate stock, Kulon Kitaika on Gallia Beauty. Work with this variety was conducted at the Mahoning County Farm.

FACTORS AFFECTING APPLE HARDINESS AND METHODS OF MEASURING RESISTANCE OF TISSUE TO LOW TEMPERATURE INJURY*

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Injury to fruit trees as a result of low temperature during late fall and winter is one of the most serious hazards in fruit production. Obviously, the loss may be particularly disturbing since such injury may not occur during growth prior to bearing but rather after some years of fruit production. Since fruit trees are comprised of at least two portions, roots and the scion variety grown for its fruit, the problem becomes doubly important. The resistance to low temperature injury of these parts may vary greatly. With the utilization of growth controlling stocks a third component may be added to the structure, a fact complicating the problem still further. Information with respect to the relative hardiness of various varieties utilized for the three portions of the tree as well as the factors affecting changes in resistance of each to low temperature injury would be particularly valuable at this time.

Since the occurrence of periods of low temperature during late fall and winter cannot be predicted in advance, it is impossible to establish experimental conditions in the orchard designed to give information relative to variations in hardiness between the components forming the tree. In consequence studies utilizing laboratory procedures to ascertain comparative resistance to low temperatures as well as the factors affecting injury must be initiated. A number of studies (19, 27, 28, 30, 35, 42, 52-54, 56, 61) have been reported in the literature which indicate the efficiency of artificial freezing methods for this purpose.

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*This work was carried out in connection with two dissertations presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State Universisty. These were:

1. Emmert, Fred Herbert.* 1952. Hardiness Studies of Apple Twigs and Blossoms.

2. Rollins, Howard Arthur Jr.* 1954. Factors Affecting the Hardiness of the Apple. Both dissertations were supported by funds supplied by State Project 50 while Rollins and Emmert were Research Assistants of the Ohio Agricultural Experiment Station.

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The experiments reported in this bulletin were designed primarily to obtain information concerning the relative resistance to low temperature of various apple varieties utilized for their fruits as well as for possible use as intermediate stocks. Certain growth regulating stocks were also included. At the same time the work was also initiated for the purpose of studying certain factors presumably affecting the degree of low temperature injury. Such information in both instances would be of inestimable value in explaining the effects of such injury under natural conditions in the orchard. Furthermore, it should permit more effective manipulation of cultural conditions in order that the hazard of injury during periods of low temperature would be greatly reduced.

REVIEW OF LITERATURE

The subject of low temperature relations in plants is complex and the literature dealing with it extensive. Excellent reviews have been prepared by Chandler (13), and Levitt (38) and should be referred to for a more complete discussion. The following review will be restricted to a brief summary of those factors which exert an influence on low temperature hardiness of apple (Malus sylvestris) and the various methods that have been employed to study the effects of these factors.

I. PLANT AND SOIL FACTORS AFFECTING THE HARDINESS OF THE APPLE

A. VARIETY

Every few years temperatures drop low enough sometime during the winter months to cause low temperature injury to apple trees. Such winters are referred to as test winters. Following each of these test winters marked differences between varieties with respect to cold hardiness have been noted and workers have attempted to evaluate apple varieties on the basis of their susceptibility to low temperature injury. (2), (8), (14), (32), (36), (40), (44), (49), (51). Although these evaluations have not always been in complete agreement, certain generalizations can be made from them. For example, the varieties Baldwin, Stayman Winesap, Rome Beauty, and Winesap have generally been reported to be more tender than Northern Spy, Golden Delicious, and Jonathan. On the other hand, such varieties as McIntosh, Yellow Transparent, Hibernal and Virginia Crab have been reported to possess a relatively high degree of resistance to low temperature injury.

B. TISSUE MATURITY

Significance of Tissue Maturity

After shoot growth has ceased, definite physiological changes take place within plant tissues which result in what has been termed "hardening off" or an increase in resistance to low temperature injury. These changes continue until maximum cold resistance is attained. The development of conditions within the tissue which result in maximum low temperature resistance is frequently referred to as "tissue maturity" in contemporary horticultural literature.

Any condition that results in a prolongation of active growth will delay the normal maturing process. While the maturing process in deciduous trees is not clearly understood, certain changes within the tissues are known to occur. Structural changes in the protoplasm, reduction in free water within the cells, increase in water-binding colloids and thus in percentage of bound water, increased sugar content of the cells with the resulting increase in osmotic pressure, are some of the more important changes taking place within the tissues during the normal maturing process.

Gourley and Howlett (29) state that the maturity of a plant and its respective tissues is one of the most important factors involved in winter hardiness. The full significance of this statement can be appreciated only after a brief survey of the more important test winters. Since 1900 severe low temperature injury to apple trees has occurred during the winters of 1903-04 (39), (59), 1906-07 (32), (49), (59), 1917-18 (14), (32), (39), (44), (59), 1925-26 (40), (43), 1933-34 (2), (8), (52), (59), 1935-36 (2), (32), (41), 1940-41 (36), (40), (45), 1947-48 (10), 1955-56 (50), and recently during 1958-59.

In all but two of the test winters since 1900 the injury was caused by low temperature conditions that occurred early in the dormant season or prior to December 15. The two exceptions are the winters of 1917-18 and 1935-36. During the winter of 1917-18 the injury resulted from a freeze that occurred on December 30. Chandler (14) stated, however, that the trees had gone into the winter in a poorly matured state due to early fall frosts and reported that the lack of tissue maturity was an important contributory factor to the injury that resulted. Oskamp (44) also attributed lack of tissue maturity as a major contributory factor to the injury that resulted from the same December 30 freeze in Indiana.

Low temperature injury during the winter of 1935-36 was reported by Anthony et al. (2) to have been caused by low temperature conditions occurring on January 22. These authors reported that after studying the effects of this cold period there appeared to be much conflicting evidence until it was realized that tissue maturity was the one factor common throughout. They state that normal maturing processes were checked by the occurrence of an unseasonable freeze in October. Havis and Lewis (32) also attributed the lack of normal maturing as a major factor in the injury resulting from this same freeze in Ohio.

A brief survey of the test winters that have occurred since 1900 reveals the fact that the one factor that appears to be common to all of these winters is the lack of tissue maturity at the time the low temperature conditions occurred.

Factors Influencing Maturity and Consequently Hardiness

Fertilization – Following the winter of 1935-36 Anthony et. al (2) noted that those trees that had received heavy applications of nitrogen were more severely injured as a result of the low temperature conditions than those trees that had been heavily fertilized. These authors state, however, that this more severe injury took place only in so far as the tree maturity or vigor had been influenced by this heavy fertilization.

The practice of fall fertilization with nitrogen has been reported as being responsible for considerable low temperature injury to apple tree trunks in New Hampshire by Tingley et al. (58) and Rawlings and Potter (47). Tingley and Potter (57) also noted that low temperature injury to trunks was more prevalent where spring applications of manure were supplied in comparison to spring applications of sodium nitrate. The authors attribute the injury to a delay in tissue maturity caused by the spring application of manure. Way (60) found that October and November applications of ammonium nitrate resulted in a significant reduction in hardiness whereas Edgerton (25) found that if ammonium nitrate were applied in late December no reduction in hardiness occurred.

Cultivation – Several workers (2), (4), (36), (44), have noted that late cultivation or any other soil management system that stimulates growth late in the season frequently results in increased low temperature injury. They attribute these efforts to a delay in the normal maturing processes of the plant.

Defoliation – Anthony et al. (2), Tingley and Potter (57) and Chandler (14) have all reported instances where apple trees defoliated by spray injury or a severe apple scab infection were more severely injured by low temperature conditions the following winter than other trees which had not been defoliated. Kennard (37) found defoliation of Montmorency sour cherry trees resulted in reduction in the cold hardiness during the following winter. Chandler (14), referring to the 1917-18 winter, and Anthony et. al. (2), referring to the 1935-36 winter, both mention that early fall frosts causing injury to the foliage affected the normal maturing processes of the trees causing the tissues to be in a poorly matured condition when severe cold periods occurred later on in the winter.

Chandler (14) attributed considerable importance to substances transported from the leaves late in the season and the influence of these substances upon the cold hardiness of the tree. He pointed out that those tissues farthest from the leaves such as those of the trunk, are the most tender, and likewise since the inner side of a branch generally possesses fewer leaves, the crotches are often tender. He also pointed out that those tissues farthest from the phloem, or in other words the pith, are the more tender.

C. SIZE OF CROP

In addition to the above mentioned factors it has been reported (39), (14), that trees bearing heavy crops of fruit are more susceptible to injury from subsequent low temperatures than similar trees bearing only moderate to light crops of fruit.A good example of this is afforded by Macoun (39), who reported that of 14 Wealthy trees, the eight which bore a crop of fruit were severely injured by low temperatures occurring the following winter whereas the six remaining trees, which had borne no fruit, were uninjured.

D. PRUNING

Many reports in the literature deal with the effect of pruning upon degree of low temperature injury. Following the freeze of January 20, 1936, Burkholder (11) reported that a number of Jonathan and Stayman Winesap trees, pruned prior to December 6, 1935, suffered more severe low temperature injury than similar adjacent unpruned trees. Blair (9) noted that in a Northern Spy orchard those trees pruned prior to a freeze on December 30, 1917 were severely injured while the remaining trees were uninjured. Anthony et al. (2) also noted that early pruning resulted in more severe injury during the winter of 1935-36 in Pennsylvania. An interesting report is provided by Gourley and Howlett (29) who relate that in an orchard in Chardon, Ohio, trees pruned just prior to a freeze in 1924 were badly injured while unpruned trees were unhurt. A similar situation was also reported by Havis (32).

E. STOCK – SCION INTERRELATIONSHIPS

Opinions vary, and conflicting results exist in connection with the reciprocal influence of stock and scion on one another with respect to winter hardiness. Filewicz (26) in Poland stated that a tender scion variety will take on the hardiness of a hardy stock within five years, and that a tender trunk may in five to ten years acquire the hardiness of hardy scions topworked upon it.

Potter (46) reported that scions of varieties of varying degrees of hardiness did not influence the hardiness of the stock roots upon which they were grafted. However, Stuart (52) found that the scion variety did exert an influence upon the hardiness of the stock but that the hardiness transmitted to the stock bore no relation to the hardiness of the scion. Waring (59) has reported that in the case of Baldwin trees topworked to McIntosh there was evidence that some of the hardiness of the McIntosh was imparted to the Baldwin trunks. Schutz and Graves (48) stated that Dolgo crab scions increased the hardiness of the Malling rootstocks I, IV, V, VII, IX, X, XIII, and XVI.

In studying the influence of a stock upon the hardiness of the scion, Stuart (52) found that in the case of one year old budded nursery stock the scion variety was not influenced by the stock as far as cold hardiness was concerned. Edgecombe (24) noted, however, that after the 1940 freeze there was less injury to the twigs of Jonathan, Turley, and Stayman when these varieties were worked on Hibernal than when grown on seedling roots. Hilborn and Waring (33) presented considerable evidence to indicate that both Hibernal and Virginia Crab interstocks imparted increased hardiness to scion varieties. These authors reported that Virginia Crab was the more effective of the two.

II. TEMPERATURE FACTORS THAT INFLUENCE THE DEGREE OF TISSUE INJURY

A. RATE OF TEMPERATURE DROP

Chandler (13) reported that a rapid drop in temperature resulted in more severe injury to twigs and buds of fruit trees than a more gradual drop in temperature, even though the tissues subjected to the more gradual drop attained a lower minimum temperature. Carrick (12) reached the same conclusion working with apple roots. These reports are strongly substantiated by Potter (46) in studies with apple seedlings. He found that if the temperature of the seedlings was depressed from room temperature to -8°C. in half an hour, more injury resulted than if the same temperature drop was extended over a period six to seven hours. Hildreth (34) in work with apple shoots obtained similar results.

B. MINIMUM TEMPERATURE

The minimum temperature to which a tissue is subjected is a very important factor in the amount of injury inflicted; however, it is by no means the only consideration. The rate of temperature fall, which was discussed above, and time of year are also important factors. Hildreth (34) established the critical low temperature at which killing occurred for the varieties Jonathan and Duchess. He found that the critical temperature in July was about -3°C. and slowly decreased to -41°C. in January. The critical temperature remained at that point until late in March or until the conditions were favorable for resumption of growth.

C. TIME AT MINIMUM TEMPERATURE

There is a definite relationship between length of time at the minimum temperature and the injury inflicted. Potter (46) showed that by subjecting seedlings to low temperature treatments of -8°C. for periods of one-half hour, eight hours, and 17 hours there was more injury to the tissues that had been subjected to the longer low temperature treatment periods. Hildreth (34) also noted that more severe injury resulted when tissues were subjected to low temperature treatment for long periods of time than for shorter periods.

D. RATE OF THAW

Carrick (12) reported, after limited tests, that the rate of thawing made no difference in the extent of injury inflicted upon apple seedling roots which had been subjected to a low temperature treatment. Potter (46) later also found that no difference in the extent of injury could be noted between roots thawed "as rapidly as possible in warm air" and roots thawed over a period of three or four hours. Hildreth (34) found, however, Wealthy apple shoots, which had been frozen for three hours at -25°C., were more severely injured when they were thawed rapidly by being plunged either in a mercury bath at +30°C. or placed under running water at +15°C. than when they were allowed to thaw more slowly. With regard to these seemingly conflicting data it should be pointed out the rapid thawing methods of Hildreth were more rapid than that employed by Potter and may account for the apparent conflicting reports.

III. MEANS OF DISTINGUISHING BETWEEN TENDER AND HARDY PLANTS

A. GENETIC CHARACTERISTICS

The problem of finding a satisfactory means by which to predict or measure the hardiness of plants has received considerable attention in the past. Many workers have attempted to find some characteristic of the apple tree which could be correlated with its resistance to low temperature injury. Many differences between hardy and tender varieties have been found to exist. Bibikova (7) found differences with respect to stomatal size while Allen (1) and Beach and Allen (5) stated that large petals were associated with hardiness yet the converse is not always true. Correlations were also found to exist between hardiness and the maturing of the wood by Allen (1) and hardiness and density of wood by Beach and Allen (5). Differences in respiration rates have been reported by Beaumont and Willaman (6) and Delong, Beaumont and Willaman (16) and likewise in dye absorption by Dunn (21), (22) and (23). Differences in the percent moisture were detected by Allen (1), Beach and Allen (5), Hildreth (34), and Stuart (52). Stuart (52) also found some correlation between hardiness and percent ash as well as between hardiness and percent dry weight. Hildreth (34) and Delong (15) found differences in pentosan content and likewise Hildreth (34) found differences in sugars and amino nitrogen.

Freezing point depression values have been found to correlate with hardiness of apple varieties to a certain extent. Bakke, Radspinner, and Maney (3), however, were not satisfied with this correlation and thus embodied the moisture determination of apple twigs in the calculation of a "hardiness factor" which is given below. These authors felt that this "hardiness factor" was more closely related to the true hardiness of a variety than freezing point depression values alone.

$$\text{Hardiness Factor} = \frac{\textbf{(freezing point depression) (\%}\mathbf{H_2O}\textbf{)}}{100}$$

At least with regard to the apple none of the previously mentioned correlations for the prediction of cold hardiness have met with any degree of success as a means of evaluating the hardiness of apple varieties. In discussing methods of determining hardiness Dunn (23) concluded, "None of the methods thus far developed can be used with certainty to determine hardiness in a practical way as a substitute for a natural or artifical freezing test." This opinion was also expressed by Hildreth (34). The remainder of this discussion will thus be devoted to natural and artificial freezing tests and methods of determining the extent of injury thereby inflicted.

B. FREEZING TESTS

Much of the information that is presently available regarding the low temperature resistance of apple tissue has been obtained from field observations following "test winters." The principal difficulty with relying upon "test winters" or, in other words, natural freezing tests for information is that they occur infrequently. Another difficulty is that the conditions prevailing during each "test winter" are often unique to that winter.

Several workers (12), (25), (28), (34), (46), (51), (52), (53), (54), (56), (60), and (61) have employed the use of various artificial freezing techniques in order to study more precisely the hardiness problem. Such methods have the advantage that the test may be executed at the discretion of the research worker. They also offer an opportunity to regulate and take into consideration the various temperature factors which influence the degree of injury as discussed previously.

IV. MEANS OF EVALUATING THE DEGREE OF LOW TEMPERATURE INJURY

In order to utilize either artificial or natural freezing tests in studying the problem of winter hardiness, it is essential that an accurate means of evaluation, as to the extent of injury, be available. One means which has frequently been usesd is to determine the extent of browning of stem tissue resulting from a low temperature treatment and thus estimating the extent of injury on this basis. Dorsey (20) utilized this means of evaluating the extent of injury resulting from low temperature conditions during the winter of 1917-18. With regard to tissue browning as means of evaluation, Dorsey (20) stated, "In the apple, the wood is severely injured before the buds are affected or the twigs show any killing back. This injury is clearly seen in the marked browning of the wood tissue, and can be detected in cases of slight injury before the vigor of the tree is perceptibly impaired. The degree of this browning varies with the severity of winter and with the hardiness of the variety and can be considered a most sensitive index of winter injury." Potter (46) as well as Wilson (62) (63) have also used tissue browning as a criterion of extent of injury.

Nichols and Lantz (43) in order to express the extent of injury more precisely formulated an "injury index." The "injury index" was calculated according to the formula $\frac{a+5b+12c+30d}{N}$, where N equals the number of trees under examination, "a" equals the number of trees with no injury, "b" the number of trees with slight injury, "c" the number of trees with medium injury and "d" the number of trees with severe injury.

Another method of evaluating the extent of low temperature injury is to study the subsequent development of tissues following low temperature treatments. Both Hildreth (34) and Swingle (56) have used this method in the evaluation of apple vegetative tissues.

In more recent years electrolytic techniques have come into use in cold hardiness studies. Since this is the approach used in the present investigation, it will be discussed in more detail.

Greathouse and Stuart (30), studying the electrical conductivity of expressed cell sap, found that the sap of a tender variety of red clover yielded a greater conductivity reading than the sap of a hardy variety. Ivanov (35), in studies with winter wheat, also utilized cell sap conductivity readings. Dexter, Tottingham and Graber (19) measured the electrical resistance of an intact tissue by placing root sections between two electrodes. These workers used alfalfa, winter wheat, and red clover in their studies and found that this method showed promise as a means of studying hardiness. Filinger and Cardwell (27) used a similar method to study low temperature injury to brambles. A few years later, Filinger and Zeiger (28) employed the same method in investigation of the cold hardiness of some French crab selections.

The most widespread use of electrical conductivity as a tool in the study of low temperature hardiness has likely been made in determining the extent of the diffusion of electrolytes from tissues subjected to low temperature treatments.* This method involves placing plant tissues, which have been subjected to low temperature treatments, in distilled water. After a given period of time the electrical conductivity of the distilled water is determined in order to discover the extent of diffusion of electrolytes from the treated tissues. Such a method is based upon the principle that low temperature injury results in a dissociation of the protoplasm within the cell. The protoplasm in this undissociated state no longer retains its differentially permeable characteristics and thus materials within the cell may diffuse outward when the injured tissue is placed in an aqueous medium. The greater the extent of the injury the greater the total outward diffusion.

The electrolytic technique has been employed by Merrill (42), Dexter (17) and Dexter, Tottingham, and Graber (18) (19) in studies with hardiness of agronomic crops. Swingle (56) investigated the possibilitise of using the electrolytic technique in apple hardiness studies. He compared results of the electrolytic technique with that of the "freeze and wait" visual method of determining injury. Complete agreement was found to exist between the two methods and the author states "conductivity readings give much the more sensitive figures for the intermediate degrees of injury." Stuart (52) (53) (54) and (55) also used the electrolytic technique as described by Swingle (56), with slight modification, in hardiness studies. The method has also been used by Hilborn and Waring (33) in a study of hardy trunk forming stocks in Maine and by Way (60) and Edgerton (25) to study the influence of cultural practices on the winter hardiness of apple trees.

MATERIALS, METHODS, AND APPARATUS

The principal objective of this investigation was to study the influence of several factors upon the low temperature resistance of apple twig tissues. To this end several individual studies were conducted and will be discussed separately. All of the trees utilized in these studies were growing at the Ohio Agricultural Experiment Station in Wooster, Ohio, Apple Creek State Farm or at the Ohio State University in Columbus, Ohio. The low temperature resistance of the trees was determined by means of artificial freezing tests carried out on twig** sections taken from these trees. The determinations were made in the

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* This technique has been refessed to as the "exosmois method" in past reports. However, since electrolytes are the primary product being tested for, and not the extent of exosmosis, it is felt that the term "electrolytic technique" would be more appropriate. This latter term is used exclusively throughout this work.

** "Twigs" refers to the axis of new growth from which leaves have abscised.

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I MATERIALS

A. VARIETAL VARIATION STUDIES

1950-51 Studies —

Low temperature tests were carried out on 55 different varieties four times during the 1950-51 dormant period*. An attempt was made to conduct hardiness tests on apple twigs at such times during the dormant period when the trees were presumed to have undergone definite physiological changes. The first series of tests was performed in autumn before the trees in the orchard were believed to have reached a fully hardened condition. (November 22-25, 1950.) The second series was performed in midwinter when the trees were presumably in a fully hardened condition and deep in the rest period** (January 11-14, 1951). A third series of tests was made later in the winter when the trees were considered to be in a fully hardened condition but were beyond the rest period (March 1-4, 1951). The fourth and last series was carried out in early spring just prior to the occurrence of bud swelling but before the advent of spring weather (March 23-25, 1951).

1953-54 Studies —

On the basis of results obtained during the course of the 1950-51 studies it was deemed advisable to investigate more thoroughly the relationships between varieties with respect to low temperature resistance over the entire dormant period of the trees. For this study nine varieties were selected: Tender — Staymared, Baldwin; Moderately hardy — Rome Beauty, Delicious, Franklin; and very hardy — Hibernal, Virginia Crab, Malus Robusta #5, and Columbia. These nine varieties were considered to represent a cross section of all apple varieties with respect to low temperature resistance.

Hardiness determinations were made on twig samples collected on November 19, 1953; December 16, 1953; January 20, 1954; February 17, 1954, and March 17, 1954 from trees of each of the nine varieties selected. The sampling dates were restricted to five because of a limited amount of suitable test material. The Baldwin, Rome Beauty, Staymared, Franklin and Delicious trees were seven years old and on domestic seedling rootstocks. The Hibernal tree was eight years old and the Malus Robusta #5, Columbia, and Virginia Crab trees were ten to twelve years old.

B. EFFECT OF INTERSTOCK STUDIES

Seven-year-old vigorous trees, originally planted to study the influence of a Hibernal interstock upon the scion variety grafted upon it, were selected for this study. The orchard was composed of six trees

* "Dormant period" refers to the time of the year when the apple tree is without leaves and visible signs of growth activity.

** "Rest Period" refers to that period of the year when the apple tree will make little to no visible growth response regardless of how favorable the environment may be.

each of a number of varieties. The six trees of any one variety were planted in succession, three of these trees being on domestic seedling roots, while the other three were grafted upon an interstock piece of the variety Hibernal. The scion variety and the presence or absence of Hibernal interstock piece were the only variables in this orchard. The varieties Baldwin, Staymared, and Delicious were selected for this study and samples were collected on the same dates as for the "Varietal Variation Study."

C. EFFECT OF VARIOUS CULTURAL TREATMENT STUDIES

Twenty-five uniform thirteen-year-old Yellow Transparent trees were used in this study. (Figure 1) They had received no differential treatments prior to the initiation of this experiment. The twenty-five trees were divided into five equal lots of five trees each. Each of the five trees within a given lot was subjected to a different treatment, with the treatmens being replicated and randomized in each of the other four lots. Samples were collected on November 5, 1953; November 26, 1953; December 18, 1953; January 13, 1954; February 19, 1954, and March 19, 1954. Each sample consisted of a composite of terminal twigs from the five trees receiving a specific treatment. The treatments were as follows:

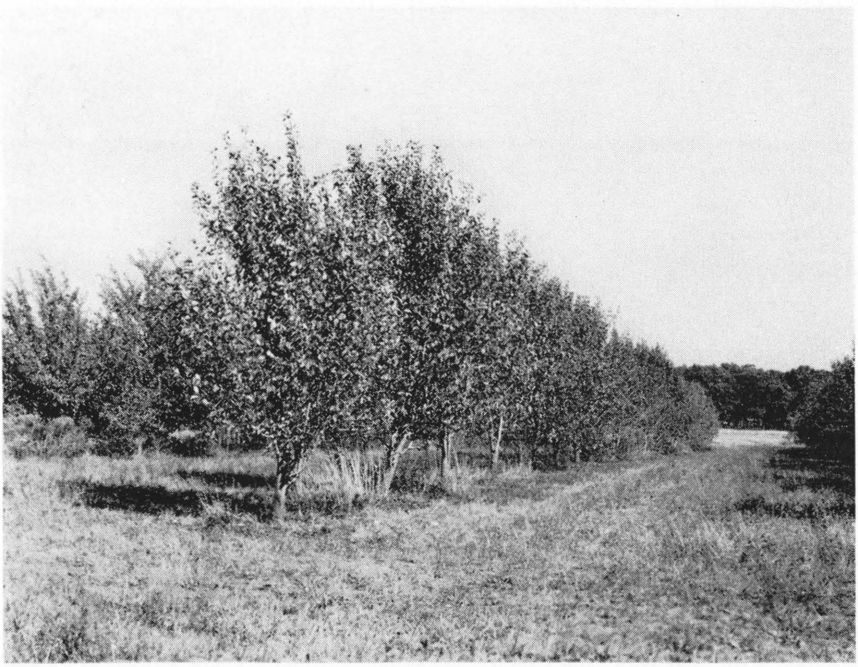

Figure 1. Yellow Transparent trees growing in the Apple Creek State Farm Orchard. September 24, 1953.

(1) "Late Nitrogen" – Two and eight-tenth pounds of a solution containing 21.7% nitrogen was applied to the area extending from approximately two feet from the trunk to the periphery of the tree on July 31st. One half of the nitrogen was in the form of ammonia nitrogen and one half in the form of nitrate nitrogen.

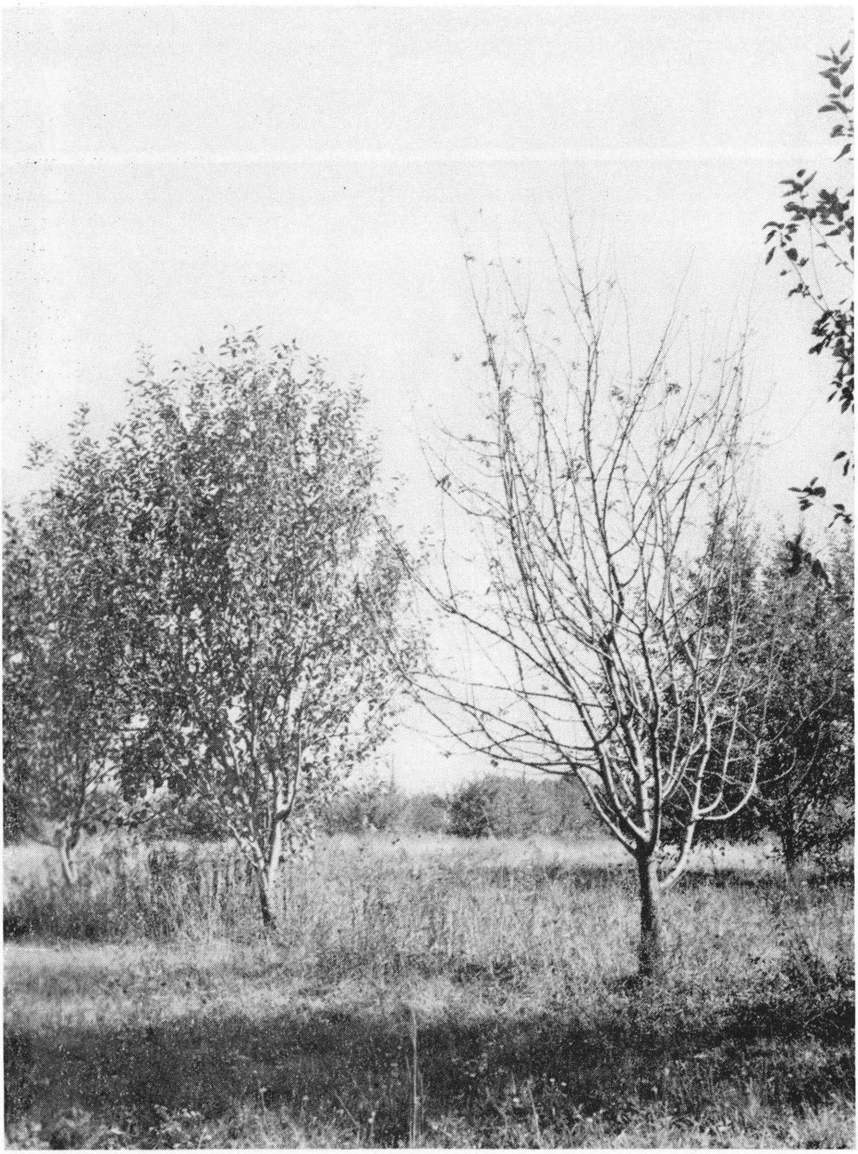

Figure 2. Defoliated Yellow Transparent tree starting to leaf out. Apple Creek State Farm Orchard, September 24, 1953.

(2) "Defoliation" – All of the leaves on the tree were removed by hand between August 17 and 19. (Figure 2).

(3) "Scoring" – Three cuts through the cambium layer continuously around the entire circumference of each of the scaffold limbs of the tree were made on August 21. (Figure 3).

(4) "Pruning" – The five trees assigned to this treatment were used in a separate pruning experiment discussed later.

(5) "Check" – These trees were subjected to no differential treatment.

A second group of trees, Golden Delicious, located in a row adjacent to the Yellow Transparent trees previously discussed, were utilized in a duplication of the above study except for the time of initiating differential treatments. The twelve Golden Delicious trees used were divided into three lots of four trees each. The treatment to which each of the four trees within a given lot was to be subjected was determined by chance, with the treatments being replicted and randomized in each of the three lots. The treatments "Late Nitrogen," "Defoliation," and "Scoring" were carried out in exactly the same manner as discussed previously except for the time of execution. The remaining tree in each set was left untreated and served as a check. The "Late Nitrogen"

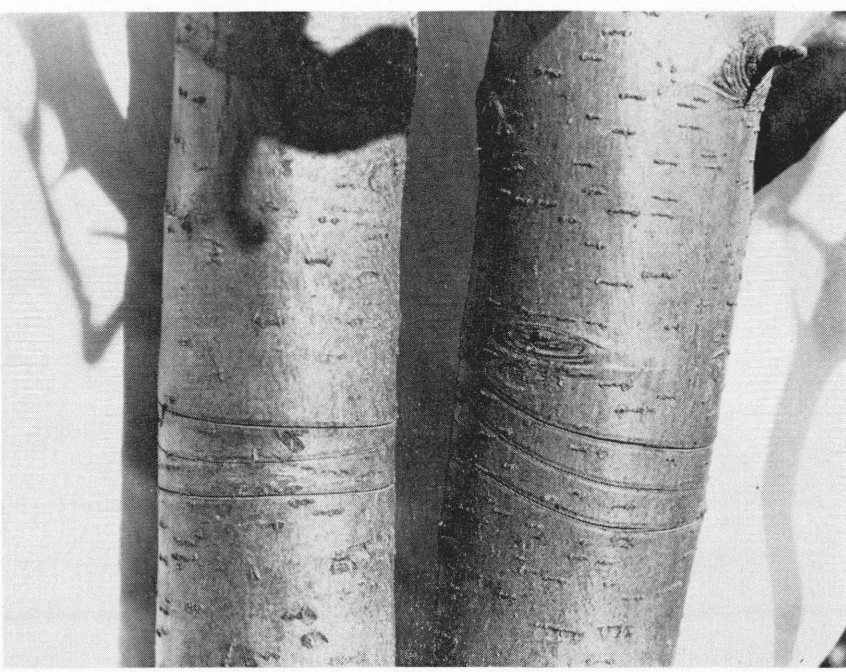

Figure 3. Scored limbs of a Yellow Transparent tree growing in the Apple Creek State Farm Orchard. September 24, 1953.

treatment was carried out on August 28, the "Defoliation" between August 27 and September 3 and the "Scoring" on September 3.

D. ROOT PRUNING STUDY

Two uniform adjacent Wolf River trees were selected to study the influence of root pruning upon low temperature resistance. One of these trees was root pruned while the other was left untreated to serve as a check. The root pruning consisted of severing all roots at a distance of thirty inches from the trunk, and to a depth of thirty inches. The point thirty inches from the trunk was approximately one-third of the distance from the trunk to the periphery of the tree. This treatment was carried out between September 14 and 16.

E. PRUNING STUDY

Four uniform thirteen-year-old Yellow Transparent trees were utilized in a study to determine the effect of pruning upon the low temperature resistance. Two of the four trees were heavily pruned between 8 and 9 a.m. on January 6, 1954. The two remaining trees were left unpruned to serve as checks. Low temperature resistance determinations were carried out on terminal twigs collected at intervals of 7 hours, 28 hours, 7 days, and 44 days after pruning from both pruned and unpruned trees.

F. MALLING STOCK STUDY

The Malling Stocks I, II, IV, V, VII, and X were compared with respect to low temperature resistance. Twig samples were collected from stool bed material growing in the nursery on the following dates: November 26, 1953; December 16, 1953; January 13, 1954; February 10, 1954; and March 19, 1954.

G. HARDINESS IN RELATION TO SEASONAL TEMPERATURE TRENDS STUDY

The Variety Golden Delicious was selected for this study because of the availability of a number of uniform trees from which to collect test material. The trees used were similar to the Golden Delicious trees used in the "Effect of Various Cultural Treatments Study" and grew in the row adjacent to them. Terminal twigs were collected on 13 different dates throughout the course of the winter. These dates are as follows:

October 18	December 9
October 25	December 16
November 5	January 6
November 12	January 13
November 19	February 10
November 26	February 19
	March 19

II METHODS

A. 1950-51 STUDIES

The 1950-51 studies were conducted in an attempt to learn more about the relative hardiness of 55 different varieties of apples at four different times during the dormant period of the trees. In these studies no attempt was made to secure data that could be used to compare the hardiness of a specific variety from one date to another other than on a relative basis. The objective was to study the relative hardiness of a number of varieties at a particular time and determine if the relative hardiness remained constant throughout the dormant period. In carrying out that objective the following experimental technique was employed.

Test materials were collected and in all instances were subjected to the desired low temperature treatment within a period of 24 hours. During the period of time intervening between collection and utilization the twigs were stored moist at 36°F.

When ready for use the twigs were cut to six inch lengths with new cuts being made at both ends of the twigs. Each twig was then individually wiped with a cloth to remove dirt, spray residue, and other foreign matter. Then approximately 40 grams of the six-inch twig sections from each variety were accurately weighed, placed in a test tube, stoppered, and subjected to the desired low temperature treatment.

In all cases the starting temperature was 40°F. and depressed at the rate of 3.5°F. $\pm$0.5° per hour until the desired minimum temperature was attained. The test tissues were then held at the minimum temperature for a period of six hours. The rate of temperature rise from the minimum following the test was not controlled but was found to be approximately eight degrees per hour.

The temperature conditions chosen for the tests were selected to similate those conditions which are ordinarily experienced in nature when a relatively low temperature suddenly follows a warm period, Minimum temperatures severe enough to cause minor injury to the tissues of the most hardy varieties tested and yet not so severe as to completely kill all of the tissues of the most tender varieties were sought. Apple twig tissues are known to be less resistant to low temperature conditions during the fall and early spring than during winter (34). For this reason minimum temperatures of minus 15°F. were employed in the fall tests, minus 50°F. in the mid and later winter tests, and minus 30°F. in the early spring tests.

The electrolytic method was employed in determining the extent to which the apple twigs had been injured. Following subjection to low temperature treatment the tubes were unstoppered and each sample of twigs submerged in 130 cc of distilled water. The samples were then left undisturbed for a period of 24 hours to allow diffusion of electrolytes from the twigs into the surrounding water to take place.

Following the diffusion period the twigs were removed from the solution and the solution volume made up to 150 cc with distilled water. The specific conductance of the solution was then determined after which the solution was discarded.* This reading was considered

* All solution conductivity determinations were made with an instrument designated as the Solu-Bridge by its manufacturers, Industrial Instruments, Inc. of Jersey City, New Jersey. **This instrument** measures specific conductance between the ranges of 10 to 1000 x 10^{-6} mhos.

to be an index of the extent to which electrolytes diffused from the test tissues as a result of low temperature injury.

Tissues were killed by adding a volume of 130 cc of boiling distilled water to each sample of twigs. Dead tissues were kept submerged in this new bath without disturbance for a period of 18 hours. The twigs were removed from the bath after this period of time and the solution volume made up to 150 cc with distilled water. The specific conductance of the solution was then tested. This reading was considered to be an index of the extent to which electrolytes were retained by the test tissues in spite of the low temperature injury. The sum of this reading plus the reading obtained earlier in the procedure was considered to be an indication of the total electrolyte content of the test tissues.

The degree of injury resulting from the low temperature treatment was then expressed in terms of precent diffusion of electrolytes which occurred as a result of the low temperature treatment injury of the total diffusion of electrolytes which would have occurred had the tissues been completely killed. These values were then used to serve as a basis of comparison of the relative hardiness of the different varieties tested.

B. 1953-54 STUDIES

On the basis of the results obtained during the course of 1950-51 studies it became the objective of the 1953-54 investigations to study the low temperature resistance of apple twig tissues over the entire dormant period as well as the influence of various factors upon this resistance. In conducting these investigations it was essential that the hardiness determinations be made in such a way that comparisons could be made between the values determined at several different dates.It was also necessary that these values be in suitable form to be employed in the development of a hardiness "curve" depicting the changes in the low temperature resistance of an individual tree or variety throughout the winter.

Previously the comparisons between individual trees or varieties have been made on the basis of evaluating the extent of injury resulting from a specific low temperature treatment. This, in essence, is what is being done whenever injury to apple trees is evaluated following a test winter. This is also the approach which was used in the 1950-51 studies and by Swingle (56), Stuart (53), and Filinger and Zeiger (28) in evaluating injury resulting from artificial freezing tests. The main objection to this means of evaluation is that if comparisons are to be made on the basis of the extent of injury resulting from a specific low temperature treatment and if the determinations were to be made at intervals over the entire season it would be necessary that the same low temperature treatment be employed in all instances. In such a case a temperature which would result in slight injury in January would by necessity be an extremely severe treatment if applied in October while a low temperature treatment which would result in slight injury in October would likely cause little or no injury if employed later in the season.

In the 1953-54 studies, in order to avoid these objections a definite amount of injury was selected and all comparisons made in terms of the low temperature treatment necessary to result in that amount of injury.

The experimental technique employed in these studies was as follows: Samples of terminal twigs were collected late in the afternoon. An attempt was always made to select twigs which would be representative of the terminal twig population of the tree or trees being sampled. The twigs were stored over night in a room which was maintained at a temperature of about 42°F. and at a relative humidity of between 85 and 90 percent. The following morning the samples were prepared for the artificial freezing test. This preparation consisted of individually wiping each twig with a damp cloth to remove any foreign matter. The twigs were then cut into 2½ inch sections. The extreme terminal and basal portions were discarded. From the sample of 2½ inch twig sections, 6 uniform, 7-gram sub-samples were weighed out and placed in vials. Five of these sub-samples were then subjected to five different low temperature treatments, and the remaining sub-sample was placed in the 42°F. room to serve as a check.

The sub-samples receiving low temperature treatments, following preparation, were placed in the test chamber. The low temperature treatment was carried out during the second night and second full day after collection of the test material. The temperature of the tissues was depressed at the rate of 3.5-4°F. per hour starting at about 35° F. This uniform rate of temperature drop was maintained until the temperature to which the first sub-sample was to be subjected was attained. The temperature was then maintained at this point for a period of two hours at which time the test chamber was opened and the sub-sample being subjected to this low temperature treatment quickly removed and placed in the 42°F. room to slowly thaw. The sub-samples remaining in the chamber were then subjected to a further depression of temperature, at the uniform rate of 3.5-4.0°F. per hour until the temperature to which the second sub-sample was to be subjected was attained. The temperature was then maintained at this point, usually 5-7°F. cooler than that to which the first sub-sample was subjected, for a period of two hours at which time the second sub-sample was removed from the test chamber and placed in the 42°F. room. This same procedure was repeated until all of the sub-samples had been subjected to the proper pre-designated low temperature treatments.

On the morning of the third full day after collection of the sample the sub-samples, including the check, were removed from the 42°F. room.The 2½ inch segments of each of theses sub-samples were cut into ½ inch segments and placed immediately in 50 cc of distilled water where they were allowed to remain undisturbed at room temperature for a period fo 24 hours. At the end of this 24 hour period the distilled water containing the materials which had diffused from the tissue segments was drained off, made up to 50 cc with distilled water, and the specific conductance of the liquid determined. The tubes containing the check segments, or those which had remained in the 42°F.

room were then replenished with 50 cc. of fresh distilled water and the mixture boiled. The check segments remained undisturbed in the distilled water for a period of 24 hours after which time the specific conductance of the distilled water containing the materials which had diffused from the segments as a result of killing by boiling was determined. From the specific conductance data, which were collected in the above manner the extent of injury expressed in terms of percent diffusion of electrolytes was calculated. An example of such data and calculations is presented in Table I.

Table I — Specific Conductance Values of Electrolytes Diffused from Baldwin Terminal Twig Samples Following Various Low Temperature Treatments.

Variety	Low Temperature Treatment in Degrees F.	A*	Specific Conductance x 10^{-5} B*	C*	D*
Baldwin	Check	13.5	- - -	46.5	- - -
"	- 6.0	15.5	2.0	- - -	4.30
"	-12.0	18.5	5.0	- - -	10.75
"	-18.0	21.0	7.5	- - -	16.13
"	-25.0	24.5	11.0	- - -	23.66
"	-32.0	27.5	14.0	- - -	30.11

*(A) Conductivity readings for electrolytes which diffused from the test materials directly following treatment.

*(B) Conductivity readings for electrolytes which diffused from test materials as a direct result of low temperature injury. (Low temperature treatment less check).

*(C) Conductivity readings for electrolytes which diffused from the checks as a result of boiling.

*(D) The percent of electrolytes which diffused from the twigs as a result of low temperature injury.

The check value in column "A" (13.5) represents the specific conductance x10^{-5} of the materials which diffused from tissue segments as a result of injury inflicted by cutting into ½ inch lengths. This value was thus subtracted from the other values in column "A" resulting in those values appearing in column "B." These values are representative of the electrolytic diffusion which took place as a result of the low temperature injury. The specific conductance value in column "C" is representative of the electrolytic diffusion which took place following killing of the check tissue by boiling. The ratio of the values in column "B" to that in column "C" results in the percentage values in column "D." These values may be considered as percent injury caused by the various low temperature treatments or more precisely the percent diffusion of electrolytes.

The percent diffusion of electrolytes values were then developed into a curve relating the extent of injury to the specific low temperature treatment. From such a curve, typified in figure 12, the low temperature treatment necessary to cause a specified amount of injury corresponding to 15 percent diffusion of electrolytes was determined. This extent of injury was used as the basis of comparison throughout this investigation and although it bears no special significance other than a convenient and consistent index upon which to base comparisons, it does correspond very closely to the amount of injury which is characterized by death of the cambium as will be shown later.

Experiments were performed in order to determine the most desirable amount of injury to employ in making comparisons. Studies were likewise made to determine the effects of sample size, segment length, length of diffusion period, as well as other details concerned with the experimental technique employed in these studies. Since many of the details of the experimental technique were based on the results of these experiments, they are included later in this bulletin and should be referred to for a more complete understanding of the method.

III APPARATUS

The success of any artificial freezing method of hardiness determination is dependent upon a temperature control unit within which tissues may be subjected to predesignated low temperature conditions. For this investigation the unit had to be of such a nature that the temperature of the test tissues could be depressed at the uniform rate of 3 to 4°F. per hour and maintained, with a tolerance of ± 0.5°F., at any one temperature for an indefinite period of time. A cross sectional view of such a unit, and the one employed in this investigation, is shown in figure 4.

The temperature control unit was divided into two compartments, one containing dry ice, which will be referred to as the refrigerant chamber, and a second larger compartment within which was located the test chamber. The entire unit was provided with a heavily insulated tight fitting cover. This cover was divided so that either of the two compartments could be opened separately. The test chamber consisted of metal tank of 36 gallon capacity. This tank rested on one-inch board strips and was removed from the walls of the compartment by a distance of 1½ inches. This insured ample movement of air over all surfaces of the test chamber.

Air circulation within the unit was provided for by a blower and a fan. (Figure 4) Fan (D) operated continuously and maintained the air surrounding the test chamber and thermostat in constant motion. Blower (A) on the other hand did not operate continuously but was regulated in its activity by thermostat (C). When in operation blower (A) drew relatively warm air from the larger compartment, passed it over the dry ice, and then discharged the newly cooled air back into the larger compartment containing the test chamber.

The temperature of the air surrounding the test chamber was governed by thermostat (C). The actual setting of the thermostat was dependent upon the degree of rotation of the outer structural shell (K in Figure 5) around an inner core or axis. By rotating this outer shell clockwise, the temperature setting of the thermostat was progressively reduced, while a counter-clockwise rotation resulted in a progressively higher temperature setting. To obtain a given temperature in the low temperature unit, the thermosat was merely rotated to, and secured at, the proper setting. If on the other hand a controlled rate of temperature drop within the unit was desired, the thermostat was slowly rotated clockwise, thus lowering the temperature setting at a gradual rate. The speed or rotation, in turn, regulated the steepness of the temperature drop.

The rotation of the thermostat at an exceedingly slow but yet uniform rate was a very sensitive operation, and was performed by the temperature control mechanism (Figure 5). This mechanism consisted of a very accurately machined cam (M) which was mounted to a time clock (N) in such a way that one complete revolution of the cam was realized every twenty-four hours. Rotation of this cam slowly forced rod (O) to the right. This rod in turn moved a lever (P) which was secured to

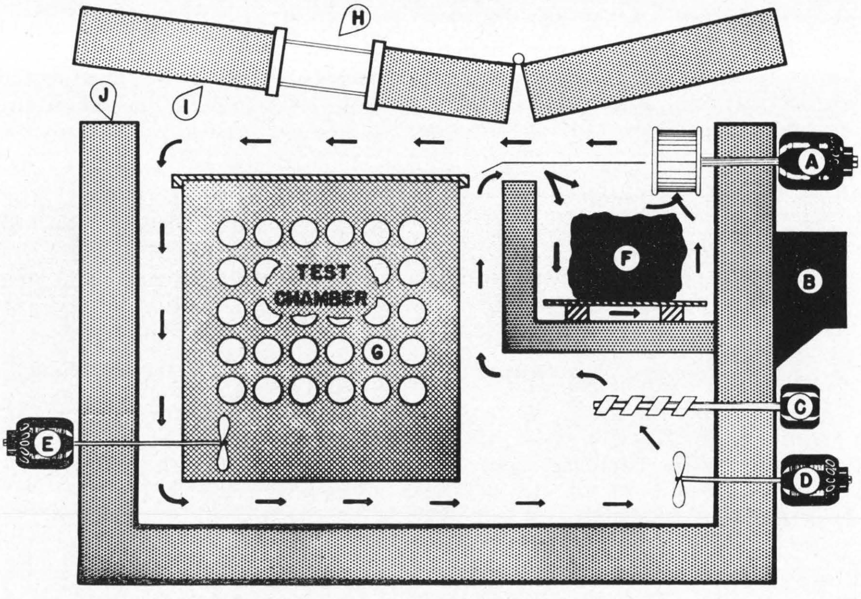

Figure 4. Cross sectional view of low temperature unit in which test materials received treatment.

A. Air blower. (thermostatically controlled)
B. Central control box
C. Thermostat.
D. Air fan. (circulating air around test chamber continuously)
E. Air fan. (circulating air in test chamber continuously)
F. Dry ice.
G. Sample vials containing test materials.
H. Observation window.
I. Insulated cover of the unit
J. Insulated walls of the unit.

Arrows indicate path of air current

the outer shell of the thermostat, and by this motion the continuous resetting of the thermostat was accomplished. The whole mechanism including time clock, cam, rod could be raised or lowered which in turn either accelerated or retarded the rate of temperature drop. If the electric time clock was stopped, the thermostat setting remained stationary and thus the temperature remained constant until the clock was started once again. A record of the temperatures within the test chamber was maintained with a Micromax (model S).*

A. 1950-51 STUDIES

In the 1950-51 studies the 36 gallon capacity test chamber tank was filled with ethylene glycol coolant. (Figure 4) A total of 72, 170 cc glass tubes containing the material to be tested were fitted into racks and then placed in the coolant filled tank for treatment.

The coolant in the tank served a twofold purpose.First, it protected the submerged plant material from blasts of supercooled air from the refrigerant chamber during the cooling process. Second, the mass of

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*Manufactured by Leeds and Northrup Company, Philadelphia, Pennsylvania.

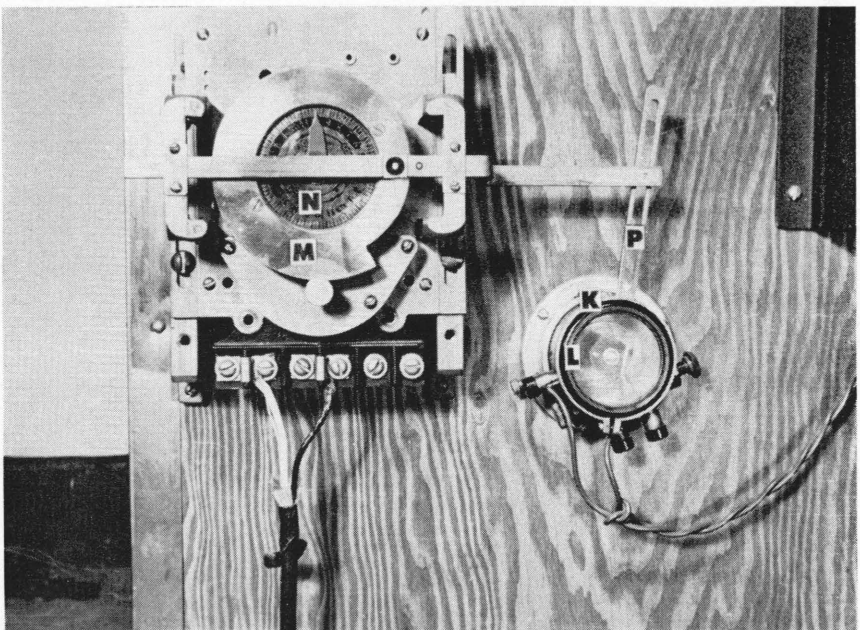


Figure 5. Temperature control unit and thermostat.

K — Outer shell of thermostat.
L — Inner core of thermostat.
M — Cam.
N — Time clock.
O — Rod which moves thermostat lever.
P — Thermostat lever.

liquid tended to smooth out any slight fluctuations in the temperature curve of the test chamber by not alowing the test materials to be influenced significantly by these small deviations.

Agitation of the coolant was provided by an electric stirrer. Thorough mixing of the liquid was further facilitated by the fact that the tubes were spaced far enough apart from one another and from the tank walls to allow an unrestrained movement of the coolant throughout any section of the tank.

In the arrangement discussed above it was found that during the period of temperature drop the temperature of the coolant would lag behind that of the air surrounding the test chamber. It was necessary to compensate for this lag. To aid in making this compensation a thermometer was placed in the coolant and a second in the surrounding air. Both of these thermometers were visible through the observation window in the temperature control unit cover.

B. 1953-54 STUDIES

Essentially the same apparatus was used in the 1953-54 studies as in the 1950-51 studies; however, modifications in the test chamber were made.The changes were made in an attempt to avoid the problems involved in using a liquid coolant in the test chamber. The most serious of these problems from the standpoint of the 1953-54 studies was the temperature lag between the liquid coolant and the surrounding air as previously discussed. Because of the technique employed in the 1950-51 studies, this lag did not present a troublesome problem. However, the technique employed in the 1953-54 studies required a more precise control of the temperature conditions within the test chamber and for this reason it was necessary that the temperature lag which resulted from the use of a liquid coolant be eliminated.

No liquid coolant was used in the 1953-54 studies. The metal test chamber was provided with a tight fitting cover which thus prevented the cooler air from the refrigerant chamber from coming in direct contact with the sample vials. The test chamber was fitted with five racks each of which held 24 sample vials. These racks were so constructed as to allow for air circulation around each of the vials and also to prevent the vials from being located too close to the walls of the test chamber. Fan (E) (Figure 4) was added to the temperature control unit following the 1950-51 studies. This fan served to maintain the air within the test chamber in continuous circulation at all times. By keeping the air in test chamber in continuous circulation, the temperature within the individual vials was found to be constant and entirely free from detectable fluctuations as well as being uniform throughout the entire chamber. The temperature conditions within the individual vials was determined and recorded by means of a Micromax with the thermocouple being located within a sample vial. After making these modifications in the low temperature unit, it was found that the temperature of the test tissues could be maintained just as accurately and uniformly as was accomplished through the use of a liquid coolant. In addition, a much more precise control over the temperature conditions within the test chamber could be maintained.

PRESENTATION OF EXPERIMENTAL DATA

To make maximum use of the electrolytics technique in apple low temperature resistance studies, a number of experiments concerned with various aspects of the technique were conducted. Many of these experiments were directly related to the modifications that were made in the methods from the 1950-51 studies to those conducted in 1953-54. These modifications were made in order that the hardiness changes of a tree or treatment might be more accurately compared from one test period to another. The results of the more important of these experiments are presented in this section along with the results of those tests concerned with the influence of various factors upon the hardiness of the apple.

I. STUDIES CONCERNED WITH THE REFINEMENT OF THE ELECTROLYTIC TECHNIQUE IN APPLE LOW TEMPERATURE RESISTANCE INVESTIGATIONS

A. VALIDITY OF METHODS AS EMPLOYED IN 1950-51 STUDIES

The techniques employed in the 1950-51 apple hardiness studies varied from those used by other workers (52, 54) in such details as

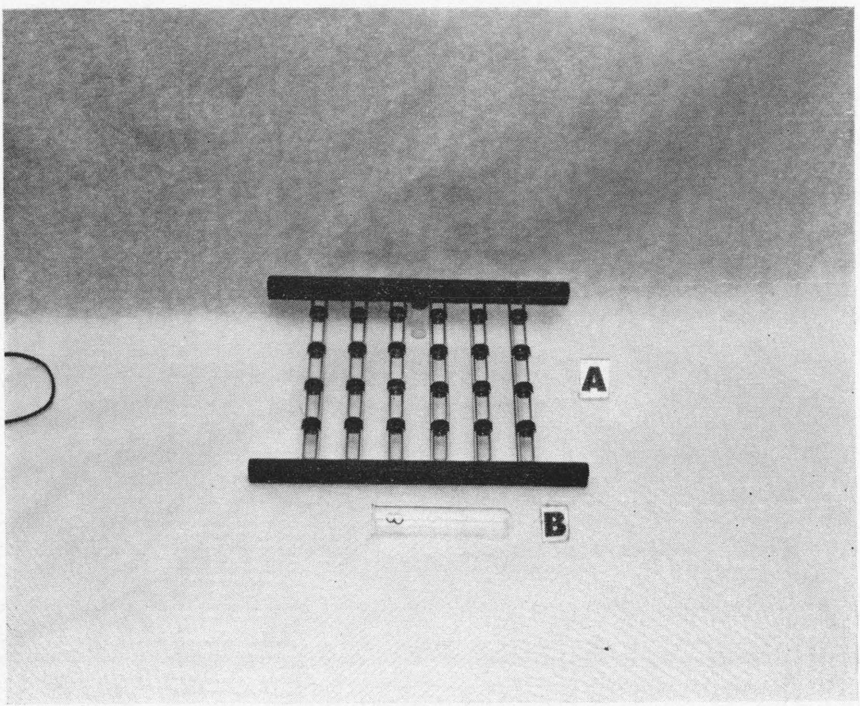


Figure 6. A. Sample vials and rack.
B. Tube within which diffusion took place.

length of twig segment, size of sample, and amount of water added to the sample. Therefore the following experiment was conducted to check the validity of the electrolytic technique as used in the 1950-51 studies.

Eighteen samples each of Hibernal, McIntosh, Stayman, and Delicious, were tested according to the methods outlined for the 1950-51 studies. The results are given in Table II, A. and B. For purposes of analysis the readings for each variety were grouped into six blocks composed of three samples each.

Table II — A. Results of the test to determine the validity of the electrolytic method as a criterion for apple twig hardiness.

	Percent Diffusion of Electroyltes			
	Hibernal	McIntosh	Stayman	Delicious
Block 1	25.52	42.24	39.01	47.38
	24.75	40.41	37.25	47.37
	23.15	40.03	36.18	47.80
Block 2	25.52	40.63	38.90	46.77
	24.02	42.55	37.40	45.47
	27.60	42.82	38.49	47.79
Block 3	28.58	40.65	39.79	48.13
	27.03	39.57	40.43	47.26
	24.43	39.36	43.21	47.21
Block 4	25.59	39.30	39.43	47.41
	22.77	37.51	40.00	46.82
	25.87	36.84	40.67	46.53
Block 5	26.97	39.11	40.81	47.19
	28.16	37.77	40.23	45.43
	27.07	39.56	37.75	46.33
Block 6	28.21	37.76	37.65	44.76
	28.15	38.27	37.66	46.83
	26.73	37.49	39.11	45.68

B. Mean values for the above data, and the differences between these means.

Variety	Mean Value	Between Means Differences
Delicious	46.79	
	)	7.24
McIntosh	39.55	
	)	0.44
Stayman	39.11	
	)	12.99
Hibernal	26.12	

Least significant difference at 5% level = 2.76

Two way analysis of variances of these data showed no significant differences between block values, indicating close agreement between replicates of the test varieties. Highly significant differences, however, were evident between the values of all varieties except McIntosh and Stayman, thus indicating the sensitivity of this technique in distinguishing hardiness differences.

B. TWIG DIAMETER

Since apple twigs vary considerably with respect to diameter the influence of twig diameter differences on the final results of the electrolytic test was studied. Twigs of McIntosh, Baldwin, and Turley apple trees were collected, separated into the three groupings outlined below, and subjected to low temperature treatment according to the method outlined for the 1950-51 studies.

Class Designation	Twig Diameter
Thin	Up to $\frac{1}{4}$ inch
Medium	$\frac{1}{4}$ to $\frac{1}{2}$ inch
Thick	More than $\frac{1}{2}$ inch

The data as presented in Table III, A and B show that the greatest amount of diffusion occurred from the thin twig samples and the least occurred from the thick twig samples. The differences in results between these two classes were statistically significant. However there was no significant difference in results between the medium size twigs and either the thick or the thin twigs.

Table III — A. Conductivity results for twigs of different diameters.

	Percent Diffusion of Electrolytes		
Variety	Thin Twigs	Medium Twigs	Thick Twigs
McIntosh	27.26	22.47	22.08
	29.39	23.91	25.94
Baldwin	27.54	29.31	23.64
	32.31	25.26	24.12
Turley	29.38	30.61	28.99
	29.43	28.75	25.03

B. Mean values for the above data, and differences beween these means.

Twig Size	Mean Value	Differences Between Means
Thin	29.22	
	)	2.50
Medium	26.72	
	)	1.75
Thick	24.97	

Least significant difference at the 5% level = 2.92

On the basis of the results obtained in the course of this experiment,, it is evident that in electrolytic hardiness tests, variations in twig diameter may be a source of discrepancy in the final test results. To reduce as much as possible this source of error a large portion of either thin or thick twigs for any given sample was avoided. Instead, an effort was always made to obtain twigs of approximately medium diameter for all samples.

C. TWIG SEGMENT LENGTH

In modifying the methods for the 1953-54 studies it became apparent that frequently the amount of available, suitable test material would be a limiting factor. For this reason tests were conducted to determine how the available test material might be most efficiently used to provide a maximum amount of hardiness information.

Uniform twigs were taken from Golden Delicious trees and divided into 28 lots each consisting of 7 grams of apple twig tissue. The principal difference between the individual 7 gram samples was the length of the twig segments. The following segment lengths and numbers were those employed:

	2.5	inches	9 segments
	2.0	inches	11 segments
	1.5	inches	15 segments
	1.0	inches	22 segments
	0.5	inches	45 segments
	0.25	inches	90 segments
(approx.)	0.125	inches	155 segments

Four samples each of the above segment lengths were prepared. Three of the four were placed in 50 cc's of distilled water and boiled to cause maximum injury. The other was placed in 50 cc's of distilled water but not boiled and served as a check. All of the samples remained undisturbed in distilled water for 24 hours at which time the specific conductance determinations were made. These data appear in Table IV and are illustrated graphically in Figure 7.

The data show that even though all samples were of the same weight and subjected to the same treatment, considerable difference was evident in the extent of diffusion of electrolytes from the individual samples. The shorter the segments, and thus the greater number of segments per sample, the greater the diffusion of electrolytes from the tissues of that sample. There was a highly significant difference between the specific conductance values for samples composed of segments of each of the different lengths studied except between the values for the $\frac{1}{4}$ and $\frac{1}{8}$ inch segment samples 90 and 155 segments per sample respectively).

The check values represent the amount of electrolytic diffusion that took place as a result of injury caused by cutting the twigs into their respective segment lengths. These values were nearly directly proportional to the number of segments per sample and thus the total cut surface area of the sample.

Table IV — **Specific conductance values for 50 cc of distilled water containing materials which diffused, over a period of 24 hours, from 7 gram samples of Golden Delicious terminal twig tissue of various segment lengths on October 6, 1953. (Length of segments indicated in terms of number of segments per sample.)**

Segments Per Sample	Specific Conductance x 10^{-5} Boiled 1	2	3	Ave.	Check	Boiled Less Checks
9	41.0	41.0	39.0	40.3	0.0	40.3
11	42.0	47.0	47.0	45.3	0.0	45.3
15	49.0	52.0	52.0	51.0	2.0	49.0
22	61.0	67.0	65.0	64.3	5.0	59.0
45	82.0	80.0	82.0	81.3	12.0	69.0
90	93.0	90.0	95.0	92.7	22.0	70.6
155	95.0	95.0	95.0	95.0	39.0	56.0
	L.S.D. at 1% level			4.9		
	L.S.D. at 5% level			3.5		

The specific conductance values for the boiled samples less those of the check samples is that portion of the total diffusion of electrolytes which took place as a result of the injury induced by boiling. These

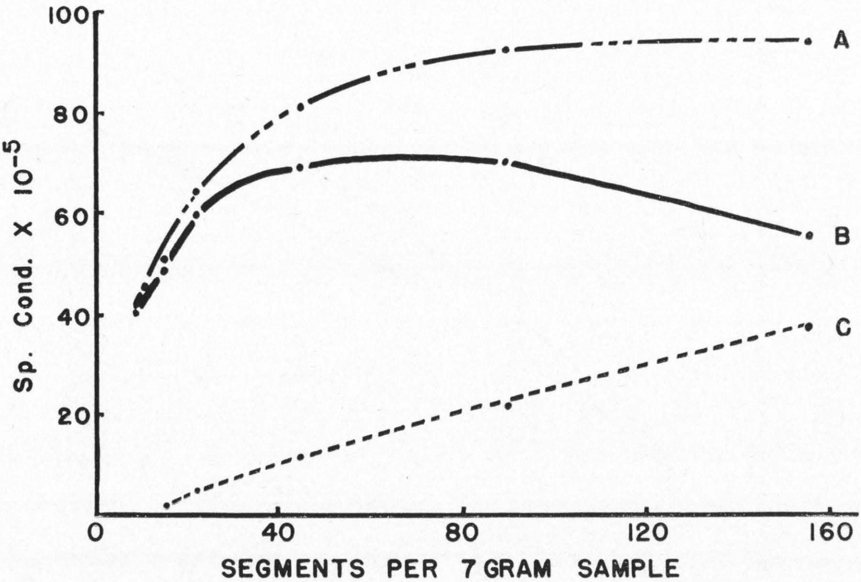


Figure 8. Specific conductance of electrolytes which diffused from 7 gram samples of successively shorter segments of Golden Delicious apple twigs on October 6, 1953. (Segment length indicated by number of segments per sample.) A— Total diffusion of electrolytes, B — total minus cut surface diffusion of electrolytes, C — cut surface diffusion of electrolytes (check).

values increased with increasing number of segments per sample to 45 segments. Little difference existed between the 45 and 90 segment per sample values because the increase in total diffusion of electrolytes was largely the result of the increase in cut surface injury. The decrease which occurred from the 90 to 155 segment per sample values was due to a considerable increase in cut surface injury. On the basis of this test ½ inch was selected as the segment length to employ during the diffusion period in the 1953-54 studies.

D. SAMPLE SIZE

In order to determine the sample size that would result in the most efficient use of suitable test material, Golden Delicious terminal twigs were collected, cut into ½ inch lengths, and divided into samples weighing 2, 4, 6, 8, and 10 grams. Four replicates of each sample size were prepared and placed in 50 cc's of distilled water. Three of the four samples in each set were boiled to cause severe injury while the remaining sample was left unboiled to serve as a check. At the end of a 24 hour diffusion period the specific conductance of distilled water containing the materials that had diffused from the samples was determined. These data are presented in Table V and illustrated graphically in Figure 8.

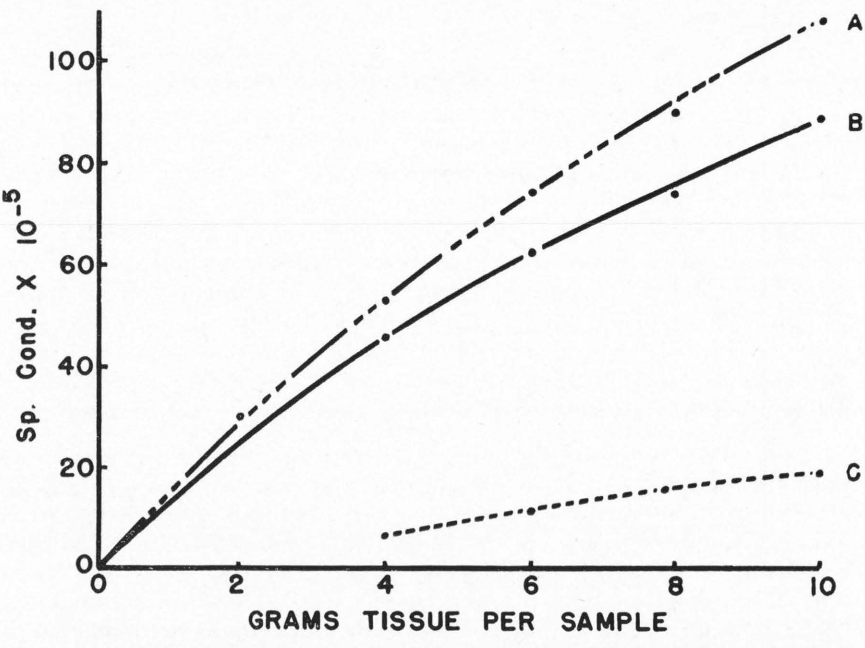


Figure 8. Specific conductance of electrolytes which diffused from various size samples of Golden Delicious twigs on September 29, 1953. A — total diffusion of electrolytes B — total minus cut surface diffusion of electrolytes, C — cut surface diffusion of electrolytes (check).

The data presented in Table V show that there was an increase in specific conductance with, but not directly proportional to, an increase in sample size. For example, the average specific conductance for 50 cc of distilled water containing those materials that diffused from 8 grams of tissue killed by boiling was not twice that of distilled water containing materials that had diffused from 4 grams of boiled tissues. It was in part of the basis of this test that 7 grams was selected as the sample size to employ in the 1953-54 studies.

Table V — Specific conductance values for 50 cc of distilled water containing materials which diffused from various sized samples of ½ inch lengths of terminal twigs of Golden Delicious over a period of 24 hours. (Test carried out on September 29, 1953).

Sample Size	Specific Conductance x 10^{-5} Boiled 1	2	3	Ave.	Check	Boiled Less Checks
2 grams	30.0	30.0	30.0	30.0	0.0	30.0
4 grams	52.0	54.0	53.0	53.0	7.0	46.0
6 grams	75.0	75.0	74.0	74.7	12.0	62.7
8 grams	89.0	90.0	93.0	90.7	16.0	74.7
10 grams	108.0	108.0	109.0	108.7	19.0	89.7
L.S.D. at 1% level				2.98		
L.S.D. at 5% level				2.05		

E. LENGTH OF DIFFUSION PERIOD

In order to study the influence of the length of the diffusion period on the amount of electrolytes diffused from injured apple twig tissues, Golden Delicious twigs were collected, cut into ½ inch lengths, divided into four similar 10 gram samples, and each boiled in 100 cc's of distilled water. Since fermentation had frequently occurred in the tube between 24 and 26 hours after the start of the diffusion period, two of the four tubes containing the apple twig segments were re-boiled 21 and 36 hours after the original boiling. Specific conductance values were determined for the solution in each of the four tubes at various diffusion period intervals up to 54 hours. These data are presented in Table VI and illustrated graphically in Figure 9.

The data show that the most rapid rate of diffusion occurred soon after the samples had been boiled with the rate decreasing markedly after 15 to 20 hours. The second and third boilings which were carried out on two of the four samples prevented fermentation and apparently had little or no effect on diffusion. The two tubes that were boiled only at the beginning of the experiment showed signs of fermentation 25 to 30 hours after boiling. This fermentation was accompanied by an increase in specific conductance. It was largely on the basis of this test that 24 hours was selected as the diffusion period for the 1953-54 studies.

Table VI — Specific conductance values of 100 cc of distilled water in which had been boiled 10 grams of ½ inch sections of Golden Delicious twigs. (Test carried out between September 22 and 25, 1953.)

Hours after Initial Boiling	Boiled at Start Only 1	2	Ave.	Boiled at Start as well as 21 and 46 hours after Start. 1	2	Ave.
1	25	25	25.0	25	24	24.5
2	30	30	30.0	30	30	30.0
3	34	34	34.0	34	33	33.5
4	37	37	37.0	37	36	36.5
6	42	42	42.0	42	41	41.5
11	50	50	50.0	50	50	50.0
21	65	65	65.0	65	65	65.0
26	70	70	70.0	70	70	70.0
31	77	79	78.0	73	73	73.0
46	84	88	86.0	76	76	76.0
54	90	95	92.5	77	77	77.0

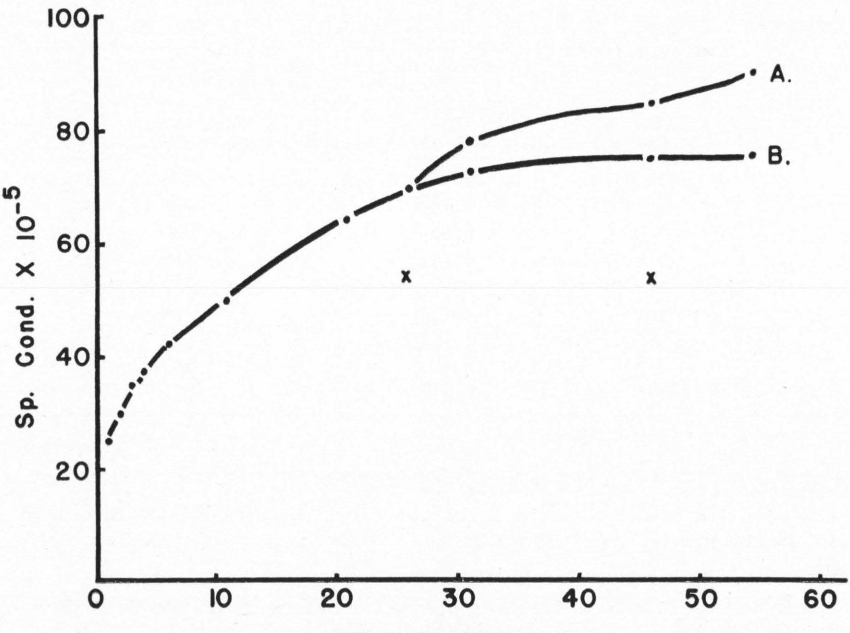


Figure 9. Specific conductance of electrolytes diffused from Golden Delicious twigs as a function of length of diffusion period. A — boiled at start only, B — boiled at points represented by "X" as well as at start.

F. COMPARISON OF KILLING BY BOILING AND BY EXTREME LOW TEMPERATURES

In order to determine what, if any, difference there might be in the amount of electrolytes which would diffuse from twig tissues killed by boiling or by extreme low temperatures, the following tests were conducted.

In connection with 1950-51 studies, 18 samples of Baldwin apple twigs were collected, prepared, and subjected to low temperature treatments according to the procedure outlined for 1950-51 studies. After the twigs which had been subjected to the low temperature treatment had regained room temperature, 9 of the 18 samples were killed by the addition of 130 cc's of boiling distilled water. The other nine samples were exposed to lethal low temperatures by being covered with chipped, dry ice for a period of two hours. The twigs were then allowed to regain room temperature and 130 cc's of unheated distilled water was added to each sample. All 18 samples remained in the solutions for 48 hours after which time the solutions were made up to 150 cc's with distilled water and conductivity readings taken. The results of this test are presented in Table VII.

Table VII — Solution conductivity readings for apple twigs killed by exposures to high temperatures, and for twigs killed by exposures to low temperatures.

Sample Number	Specific Conductance x 10^{-5}	
	Twigs Killed by Boiling Water	Twigs Killed by Dry Ice
1	1.542*	1.082*
2	1.460	1.148
3	1.435	1.140
4	1.142	1.079
5	1.067	1.032
6	1.132	1.070
7	1.151	1.364
8	1.178	1.587
9	0.910	1.377

*Calculated on a gram fresh weight basis.

No statistically significant difference was found to exist between the results of the two treatmens at the 5% level of probability, and thus it can be assumed that both methods of killing gave like conductivity results.

Prior to the 1953-54 studies, a test similar to that just described was performed using a slightly different technique. Twenty uniform 7-gram Golden Delicious samples were collected and placed in vials. Ten of these twenty samples were surrounded with chipped, dry ice for 24 hours. The temperature within the vials during this 24-hour period was minus 100 degrees F. or lower. The other ten samples remained in a 42 degree F. room during this 24-hour period. At the end of this period all samples were cut into one-half inch segments and placed in

50 c's of distilled water. The ten tubes containing the samples that had been stored in the 42°F. room were then boiled. All 20 samples remained undisturbed for a period of 24 hours, at the end of which time specific conductance determinations were made. The results are presented in Table VIII.

Table VIII — Specific conductance x 10^{-5} values of 50 cc of distilled water containing materials which diffused from 7 gram samples of 1/2 inch Golden Delicious terminal twig segments, collected on March 21, 1954, in 24 hours following severe boiling and low temperature treatments.

Sample	Treatment	
	Boil	-100°F.
1	80.0	74.5
2	78.0	75.0
3	75.0	76.5
4	77.0	74.5
5	79.5	78.0
6	79.5	79.0
7	76.0	75.0
8	76.5	81.0
9	76.5	78.0
10	76.5	78.5
Ave.	77.45	77.00

No significant difference at 5% level.

The data show no significant difference at the 5% level of probability between the amount of electrolytes which diffused from twigs killed by boiling or by extreme low temperature treatment.

G. TIME AT MINIMUM TEMPERATURE

To determine the influence of the length of time at the minimum temperature upon the injury caused Golden Delicious, terminal twigs were collected and prepared for treatment according to the procedure outlined for the 1953-54 studies. A total of 45 uniform samples were employed with five replicate 7 gram samples being subjected to each of 8 different treatments. The treatments consisted of 0, 1/2, 1, 1 1/2, 2, 3, 4, and 6 hours at minus 18 degrees F., while the check tissues were stored in the 42 degree F. room. Following treatment, the injury resulting was evaluated according to the electrolytic technique as previous described. The results are summarized in Table IX and illustrated graphically in Figure 10.

The data show a small but significant increase in injury, expressed in percent diffusion of electrolytes, with increases in the length of time that the tissue had been subjected to the minimum temperature. This increase occurred at a more rapid rate during the first part of a low temperature treatment peiod than late in the period. For example, there was a highly significant increase of 3.24 percent diffusion of elec-

Table IX — Injury to Golden Delicious terminal twig tissues collected on March 21, 1954 as influenced by various periods of exposure to minus -18°F. and presented in terms of percent diffusion of electrolytes.

Exposure Period in Hours	Percent Diffusion of Electrolytes (Ave. of 5 replicates)
0*	10.04
½	11.22
1	12.55
1½	13.14
2	13.28
3	15.21
4	14.62
6	15.95
L.S.D. at 1% level	2.607
L.S.D. at 5% level	1.831

* Sample subjected to minimum temperature but not allowed to remain for any period of time at that temperature.

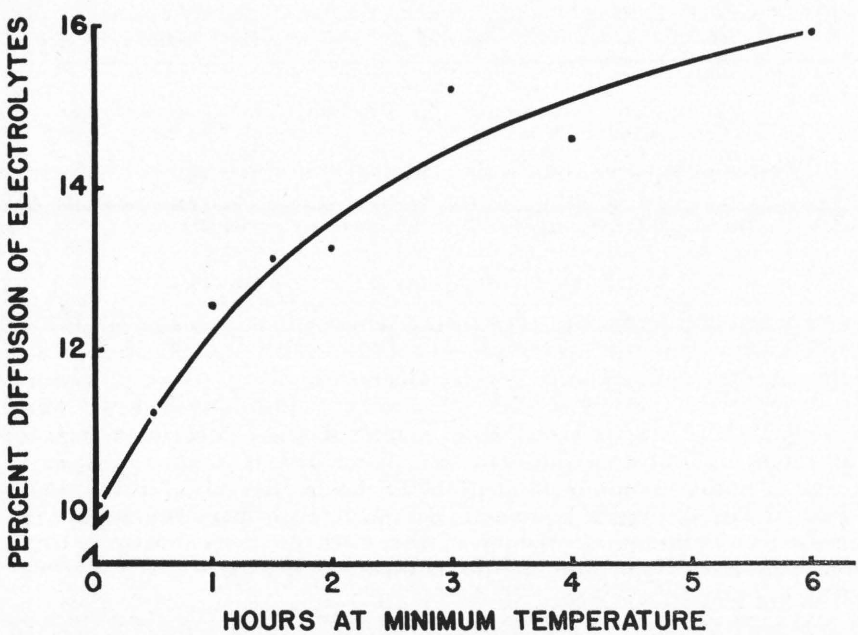


Figure 10. The percent of electrolytes diffused from Golden Delicious twigs collected on March 21, 1954 as a function of the length of time these tissues were maintained at the minimum temperature.

trolytes during the first two hours that the tissues were subjected to the minimum low temperature treatment. On the other hand, the percent diffusion of electrolytes increased only 1.33 percent from the 4th to the 6th hour of subjection to the minimum temperature. This difference is not significant at the 5 percent level of probability. It was in part based on the results of this test that two hours at the minimum temperature was selected as the interval to be employed in the 1953-54 studies.

H. COMPARISON OF PERCENT DIFFUSION OF ELECTROLYTES AND EXTENT OF TISSUE BROWNING

To study the significance of a given "percent diffusion of electrolytes" in relation to visible injury, 30 uniform 7-gram Golden Delicious twig samples were collected and subjected to 6 different treatments according to the procedure outlined for the 1953-54 studies. The six treatments consisted of five low temperature treatments -10, -05, -11.0, -20.0, -30.0, degrees F. in addition to a check treatment of storage in a 42 degree F. room. The injury resulting from these treatments were evaluated on the basis of percent diffusion of electrolytes. A summary of these data are presented in Table X and illustrated graphically in Figure 11.

Table X — Injury to terminal twigs of Golden Delicious caused by various low temperature treatments and expressed in terms of percent diffusion of electrolytes. November 12, 1953.

Low Temperature Treatment	Percent Diffusion of Electrolytes (average of 5 values)
10.0°F.	0.00
0.5°F.	4.52
-11.0°F.	14.01
-20.0°F.	26.81
-30.0°F.	43.98
L.S.D. at 1% level	3.635
L.S.D. at 5% level	2.593

A second set of samples, identical to those just described, were subjected to the same six treatments. These samples, however, were not placed in distilled water but rather held for four days after thawing at which time microscopic examinations of the internal tissues were made to determine the extent of browning. Since both sets of samples were similar in nature and were both subjected to the same treatments, the two methods of injury evaluation could be compared.

The internal tissues of the twigs that had been subjected to the low temperature of 10°F. showed no visible signs of injury. Specific conductance data also gave no indication of injury. These samples could not be distinguished from the check samples by either method of injury evaluation. Those tissues subjected to the low temperature treatment of 0.5°F. showed slight browning of the innermost xylem, while the conductivity data indicated that the injury caused by this treatment had resulted in 4.52 percent diffusion of electrolytes. The low temperature treatment of -11°F. caused injury exemplified by a browning of the pith and severe browning of the innermost xylem grading off to slight browning of the outermost xylem. The cambium also showed signs of injury as indicated by a slight browning of small areas of this layer. There was no visible injury to the phloem. This amount of injury corresponded to 14.01 percent diffusion of electrolytes. Tissues that had been subjected to -20°F. showed marked injury with severe browning of the pith, xylem, and cambium and signs of slight injury to the phloem. This degree of injury corresponded to 26.81 percent diffusion of eletcrolytes. A low temperature treatment of -30°F. caused severe browning of the pith, xylem, and cambium. Most of the phloem was also browned with the remaining areas exhibiting a watery translucent appearance. This degree of injury corresponded to 43.98 percent diffusion of electrolytes.

It was largely on the basis of this experiment that the decision was made to base all hardiness comparisons in the 1953-54 studies on the low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes. In the selection of this value it was not intended to attach undue significance to it. Nevertheless it does correspond very closely to the point at which the cambium layer was killed which is one of the principal reasons that it was selected.

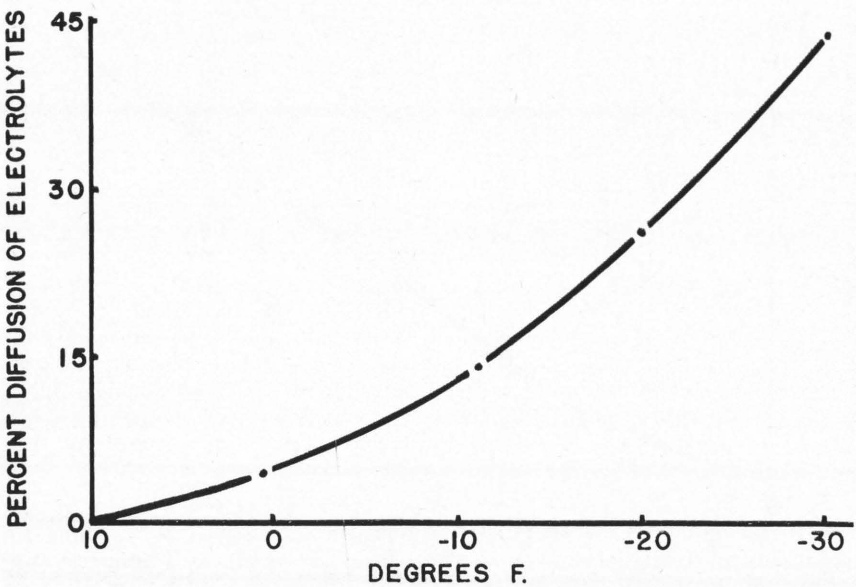


Figure 11. The percent of electrolytes diffused from Golden Delicious twigs collected on November 12, 1953 as a function of decreasing low temperature treatments.

I. SENSITIVITY OF METHODS

If hardiness comparisons are to be based on the low temperature treatment necessary to cause a specific amount of injury it is essential that the methods of low temperature injury evaluation be extremely sensitive. To determine if the methods developed for the 1953-54 studies were sufficiently sensitive to allow such comparisons to be made the following test was conducted. Golden Delicious terminal twigs were collected, divided into 30 uniform 7-gram samples and subjected to 6 different treatments. The treatments consisted of 15°F., 10°F., 5°F., 0°F., and —5°F. and a check treatment of storage at 42°F. Low temperature treatments and injury evaluations were carried out according to the procedure outlined for the 1953-54 studies. A summary of the data is presented in Table XI and illustrated graphically in Figure 12.

There was a highly significant difference between the extent of injury that resulted from each of the five low temperature treatments. The increase in the injury caused by each 5°F. drop in low temperature treatment in all but one instance was approximately twice that necessary to be significant at the 1 percent level of probability. On the basis of this test the methods used were considered to be sufficiently sensitive to be employed in the 1953-54 studies.

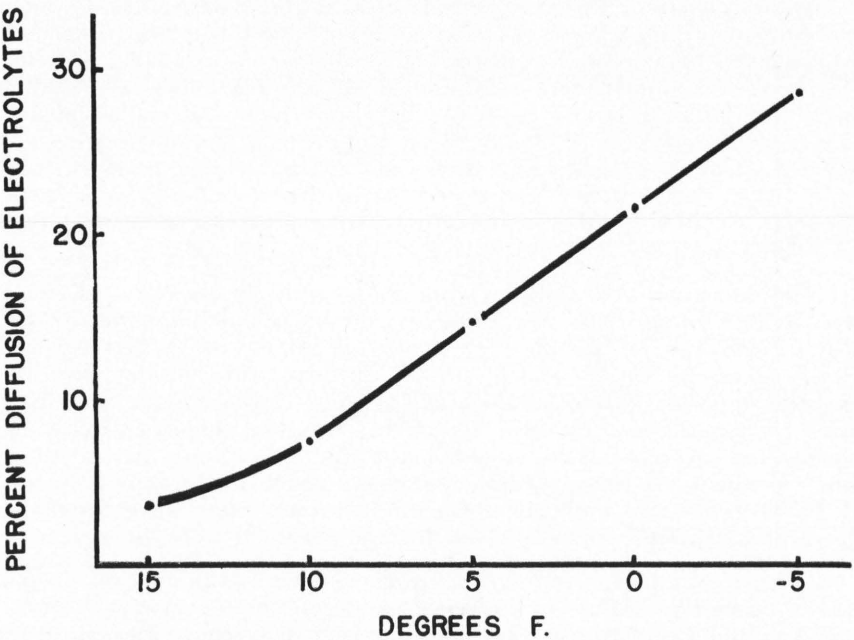


Figure 12. The percent of electrolytes diffused from Golden Delicious twigs collected on October 29, 1953 as a function of decreasing low temperature treatments.

Table XI — Injury to terminal twigs of Golden Delicious, collected on October 29, 1953 caused by various low temperature treatments and expressed in terms of percent diffusion of electrolytes.

Low Temperature Treatment	Percent Diffusion of Electrolytes (average of 5 values)
15.0°F.	3.62
10.0°F.	7.56
5.0°F.	14.80
0.0°F.	21.71
- 5.0°F.	28.62
L.S.D. at 1% level	3.534
L.S.D. at 5% level	2.565

II. HARDINESS AS INFLUENCED BY VARIOUS FACTORS

The hardiness of the apple is known to be influenced by many factors both genetic and environmental. The following test were conducted in an attempt to learn more about the hardiness of he apple and those factors influencing it.

A. VARIETAL VARIATION STUDY

1950-51 Studies

Apple varieties may vary considerably in their resistance to low temperature injury. New varieties are continually being collected from areas of harsh winter climates such as Russia and Canada. These varieties are frequently of value in fruit breeding programs where hardiness is an important consideration. This study was undertaken in an attempt to examine more closely the hardiness of some of these newer varieties as compared with some of the more popular, well known ones. Apple twigs were gathered and tested for hardiness at four consecutive test periods starting with fall according to the procedures outlined for the 1950-51 studies.

The conductivity results showing the percent of the total electrolytes which diffused from the sample as a result of low temperature injury, are presented in Figure 13. The range for any single test period extends from the variety which showed the least diffusion for the period (the most hardy one) to the variety which displayed the most diffusion for that period (the most tender variety), and these two extreme readings for each period are noted in the figure heading. All calculations are thus on a relative basis, making it possible to compare varieties between as well as within test periods, even though different temperature conditions were employed for the different periods.

The test results for fall agreed quite well with contemporary concepts concerning hardiness differences between apple varieties. Bedford, Virginia Crab, Antonovka Shafran, Anaros, Garnet Crab, Beauty Crab, Columbia, Osman, Hibernal, Tayezhnoie, Pioneer, Mecca x Dolgo, Yellow Transparent, Robin, Antonovka, and Malus Robusta #5 are considered to be extremely hardy and were so noted in the fall re-

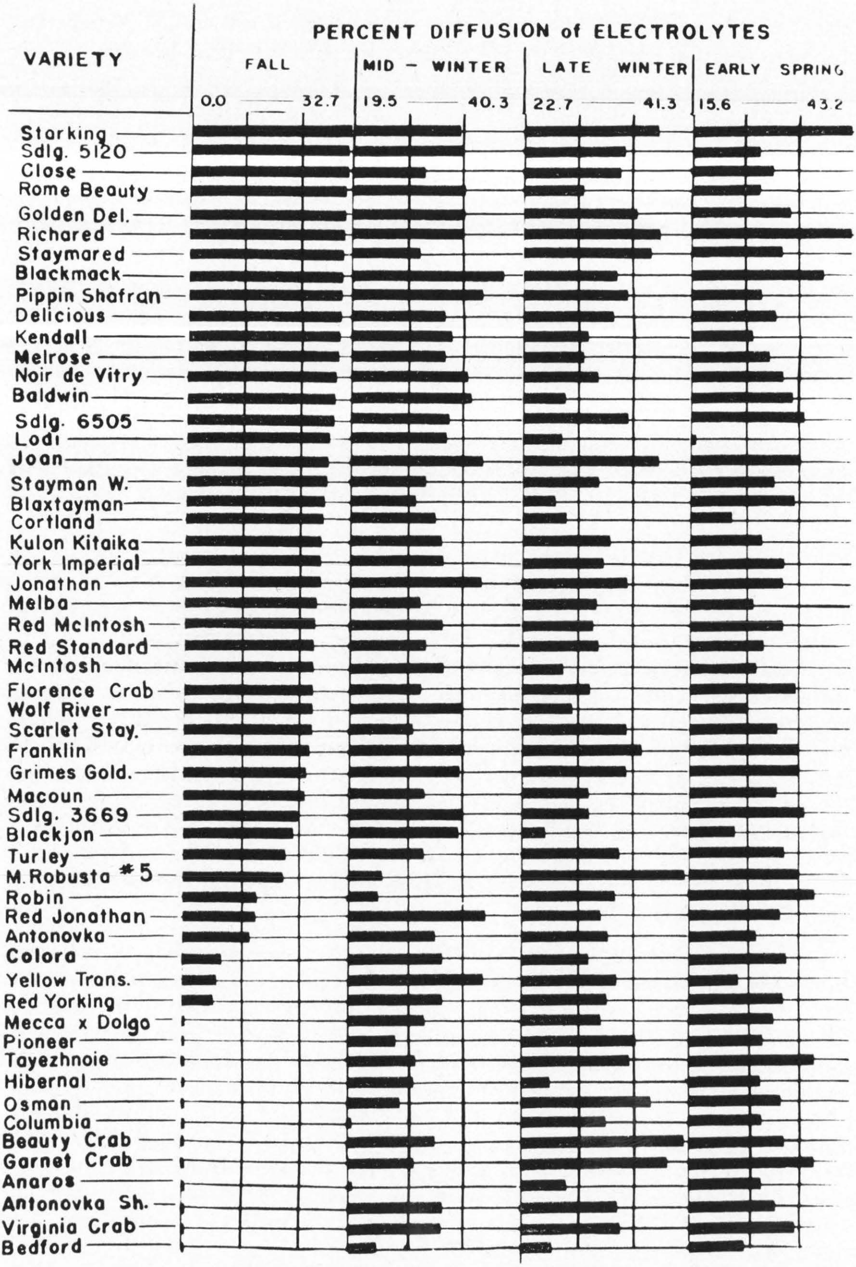


Figure 13. Hardiness ratings throughout the dormant season of fifty five varieties of apple twigs, based on electrical conductivity determinations. The two extreme readings for each of the four test periods are given in the heading. Del. — Delicious; W. — Winesap; Stay. — Staymared; Gold. — Golden Delicious, Trans. — Yellow Transparent; Sh. — Shafran; Seedling 5120 — Gallia x Red Spy; Seedling 6505 — Ingram x Delicious; Seedling 3669 — Ruby.

sults. Cortland, York Imperial, Jonathan, McIntosh, Wolf River, and Macoun are considered to be of approximately medium hardiness, and the results obtained with these varieties in the fall seemed to verify this conclusion. Also, Baldwin, Delicious, Starking, Richared, Staymared, and Golden Delicious, commonly considered relatively tender, were noted as tender in the fall test. However, a few inconsistencies were also evident. Stayman Winesap and Blaxtayman, usually considered to be relatively tender, were of medium hardiness in this test. Rome Beauty, Blackmack, Kendall, and Noir de Vitry, noted as relatively tender in the test, have generally been considered to be of at least medium hardiness, while Kulon Kitaika, Red Standard, and Florence Crab, noted as medium hardy, have generally been considered to be of outstanding hardiness.

As indicated in Figure 13 the relative hardiness status of most varieties changed considerably with time, so that the results for later test periods resembled very little the fall results or the contemporary concepts just mentioned. These changes did not, as a whole, appear to follow a set pattern, but it was possible to group certain varieties together on the basis of these changes. For instance, practically all the varieties of outstanding hardiness in the fall tests assumed a relatively more tender position with time. Virginia Crab, Pioneer, Antonovka Shafran, Garnet Crab, Beauty Crab, Osman, Tayezhnoie, Mecca x Dolgo, Red Yorking, Colora, Red Jonathan, and Yellow Transparent changed rapidy to a comparatively more tender condition, and therefore experienced a considerable decrease in hardiness (with regard to the other varieties) as the season progressed. However, Bedford, Anaros, and Columbia were slow to change in this respect and were still relatively hardy late in the dormant season. The direction of change for Malus Robusta #5 and Robin was inconclusive since both varieties displayed an initial increase in relative hardiness from fall to mid-winter, but in late winter and early spring rapidly decreased in low temperature resistance.

The more tender varieties in the fall displayed an entirely different change in relative hardiness from that of the initially very hardy varieties just considered. These more tender varieties generally increased in comparative hardiness with time. Such a change was clearly illustrated by Lodi which was noted as one of the most tender varieties in fall and the most hardy variety by early spring. Other varieties which were conspicuous in this change were Close, Rome Beauty, Pippin Shafran, Delicious, Kendall, Melrose, Cortland, Melba, Red Standard, McIntosh, Wolf River, and Blackjon. Varieties which also manifested this trend toward hardiness, but less prominently, were Golden Delicious, Noir de Vitry, Stayman Winesap, Kulon Kitaika, York Imperial, Jonathan, Red McIntosh, Macoun, Joan, and Florence Crab.

A few varieties including Starking, Richared, Blackmack, Franklin, Grimes Golden, Turley, and Antonovka, representing various hardiness classifications in fall, changed very little in relative hardiness throughout the season.

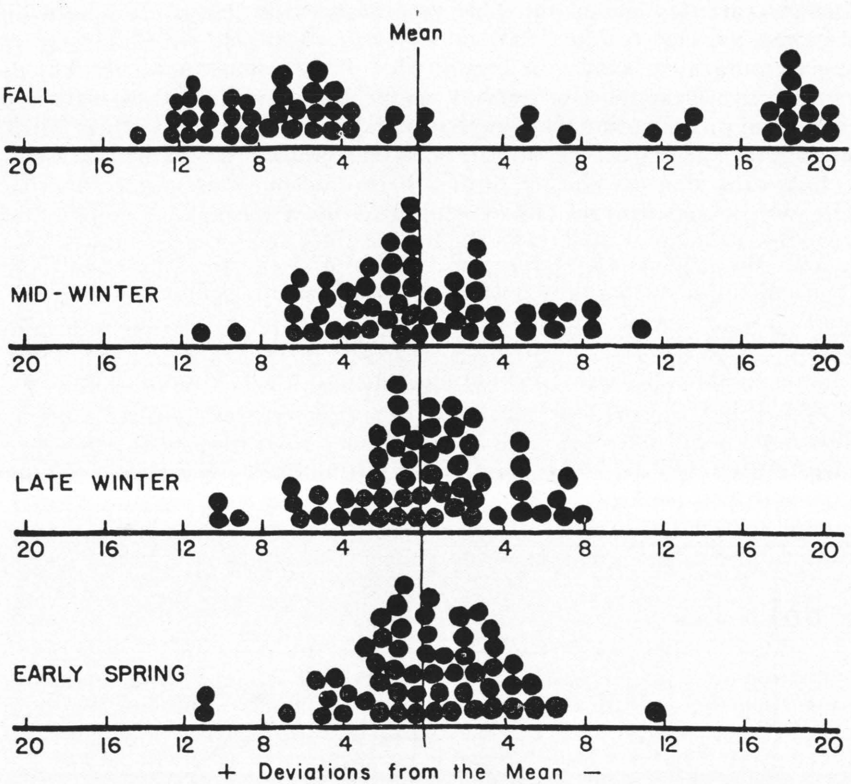
The fluctuations with time in the relative hardiness status of the various varieties is illustrated in yet another way by grouping the individual varietal conductivity values for each period around their respective means as shown in Figure 14. The variance quantity was derived for each of the test periods, and a comparison of these values to note if significant changes occurred with time in the distribution of the individual varietal hardness values, is also shown in Figure 14. The results show that a wide spread or distribution of values occurred in the fall with a considerable difference between the most hardy and tender varieties evident at that time. The hardiness differences between varieties diminished considerably by mid-winter, so that the corresponding mid-winter variance was significantly different satistically from the fall variance. The values tended to cluster still more tightly around the mean as the season progressed beyond the mid-winter period, but these later changes were not statistically significant. These data thus indicate that the greatest hardiness differences between these varieties occurred in the fall, and that as the season progressed such differences largely disappeared. The greatest change toward a common hardiness occurred between fall and mid-winter. By the end of the dormant season only comparatively small differences remained in hardiness between most of the varieties tested.

1953-54 Studies

These studies were undertaken in order to ascertain more precisely variations in the relative hardiness of varieties depending upon the time that the hardiness determination was made. The low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes was determined for the nine varieties at five different times during the winter according to the procedures outlined for the 1953-54 work. These low temperature treatment values are presented in Table XII and illustrated graphically in the form of hardiness curves in Figures 15 and 16.

Table XII — Low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from the terminal twigs of nine varieties of apples collected at various dates.

	Temperatures Required to Result in 15 Percent Diffusion on				
Variety	Nov. 19	Dec. 16	Jan. 20	Feb. 17	Mar. 17
Staymared	- 0.0	-15.6	-29.3	-23.0	-21.7
Delicious	-11.5	-26.5	-34.0	-29.0	-23.0
Baldwin	- 7.0	-20.8	-29.9	-26.0	-16.8
Rome Beauty	- 7.5	-23.8	-30.2	-30.0	-17.0
Hibernal	-29.2	-43.5	-45.6	-40.0	-28.8
Franklin	-18.7	-31.6	-39.0	-30.2	-24.5
Columbia	-33.6	-47.8	-43.5	-29.6	-21.7
Virginia Crab	-26.6	-45.2	-44.3	-30.5	-28.0
Malus Robusta #5	-22.7	-47.0	-42.8	-27.3	-16.0



	Mid Winter Variance	Late Winter Variance	Early Spring Variance
Fall Variance	*6.61	* 7.32	* 7.98
Mid Winter Variance		1.10	1.20
Late Winter Variance			1.08

*Significance at 5% level = 1.71
" " 1% " = 2.04

Figure 14. (Top) Distribution of the individual varietal conductivity values around their respective means for each test period, and (Bottom) a comparison of these distributions according to Snedecor's Test for Homogeneity of Variances.

The data show that the difference between the hardiness of the twigs of the most hardy and most tender varieties was greater early in the dormant season than later on. This is illustrated by the fact that on November 19 there was a difference of 33.6 degrees F. in the low temperature treatment necessary to cause the same degree of injury in Staymared as in Columbia; whereas on March 17 there was only a difference of 12.8 degrees F. in the low temperature treatment necessary to cause the same degree of injury in the most tender variety on that date, Malus Robusta #5, and the most hardy, Hibernal.

Of the nine varieties studied, Staymared was the most tender followed in order of increasing hardiness by Baldwin, Rome Beauty, Delicious, and Franklin. These five varieties occupied the same relative position with respect to hardiness through February 17th. The other four more hardy varieties, Columbia, Virginia Crab, Hibernal, and Malus Robusta #5 increased in hardiness over the same period during which the other varieties decreased. In general the variety Hibernal was slightly more hardy over the entire dormant season than any of the other varieties studied.

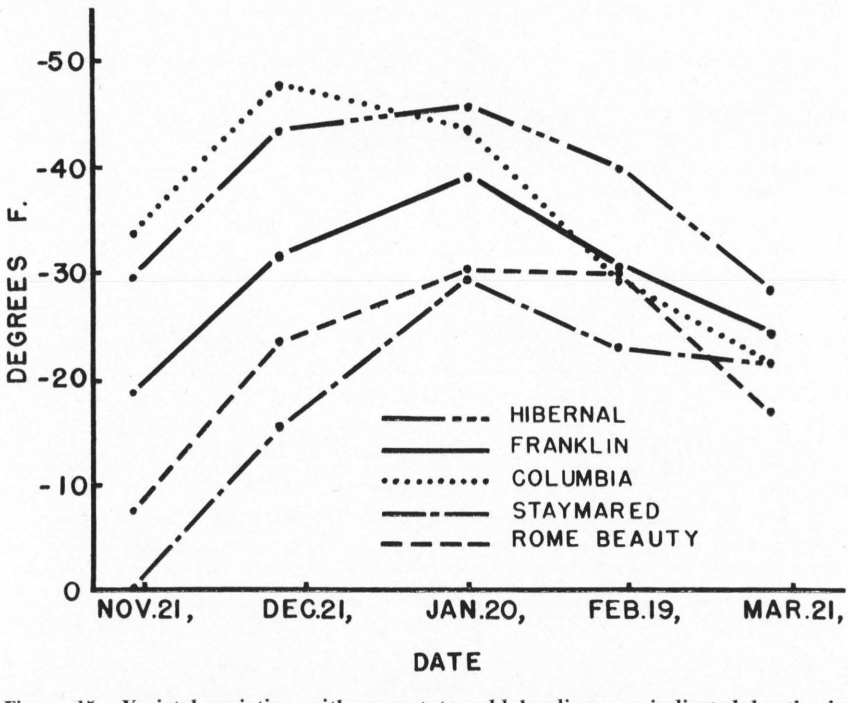


Figure 15. Varietal variation with respect to cold hardiness as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates. (Graph 1).

The data also indicate that the hardiness of Malus Robusta #5 increased more rapidly in the fall and decreased more rapidly from January 20 to March 17 than any other variety tested. On December 16 it was exceeded only by Columbia in hardiness, whereas on March 17 it was the most tender variety studied.

B. EFFECT OF INTERSTOCK STUDY

Terminal twigs from Staymared, Delicious, and Baldwin trees on Hibernal interstocks as well as similar samples from the same three varieties on domestic seedling roots were collected. The cold hardiness of the twig tissues was determined according to the procedure outlined for the 1953-54 studies with the resulting low temperature treatment values being presented in Table XIII. Those trees on domestic seedling roots served as checks.

Terminal twigs from Staymared trees on Hibernal interstocks were slightly more hardy than similar Staymared twigs from trees on seedling roots on each of the five test dates (Figures 17). Generally, similar results were attained for the variety Baldwin (Figure 18), but there was little or no difference in the cold hardiness of Delicious twigs from trees on the two different stocks (Figure 19).

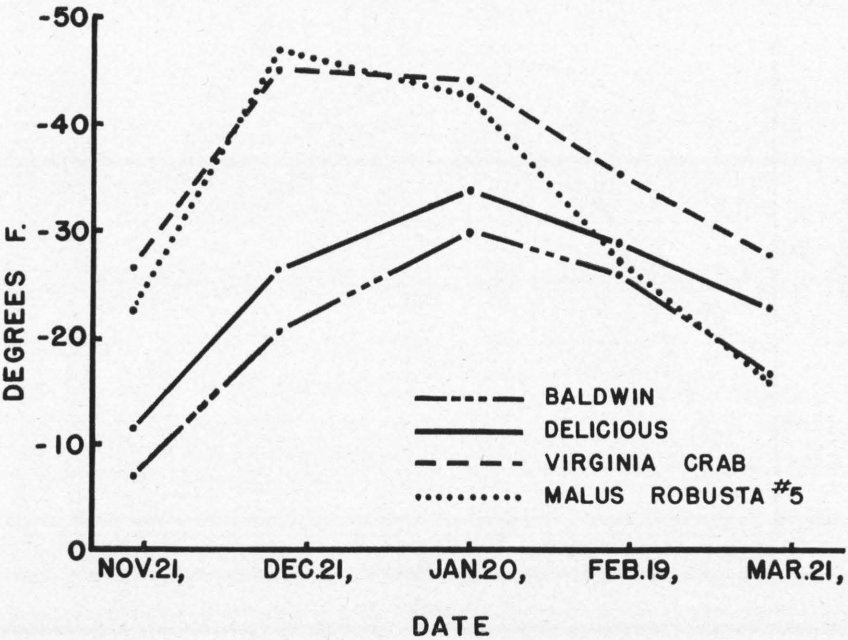


Figure 16. Varietal variation with respect of cold hardiness as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates. (Graph 2).

Table XIII — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of three varieties each on two different stocks collected at various dates.

		Temperature in Degrees F. Required to Result in 15 Percent Diffusion on				
Variety	Interstock	Nov. 19	Dec. 16	Jan. 20	Feb. 17	Mar. 17
Staymared	Hibernal	- 3.5	-23.3	-32.0	-26.5	-23.4
Staymared	None	0.0	-15.6	-29.3	-23.0	-21.7
Delicious	Hibernal	- 7.5	-26.7	-35.0	-30.0	-23.0
Delicious	None	-11.5	-26.5	-34.0	-29.0	-23.0
Baldwin	Hibernal	- 6.0	-23.8	-30.0	-29.5	-19.7
Baldwin	None	- 7.0	-20.8	-28.8	-26.0	-16.8

C. CULTURAL TREATMENT STUDY

The influence of several cultural treatments on the resistance of apple trees to low temperature injury was studied at six different dates during the dormant season. The treatments, late nitrogen, scoring, and defoliation as previously described were carried out on Yellow Transparent trees and later repeated on Golden Delicious trees. Hardiness determinations were made according to the procedure outlined for the 1953-54 studies. The results of these determinations are summarized in Table XIV and illustrated graphically in Figures 20 and 21.

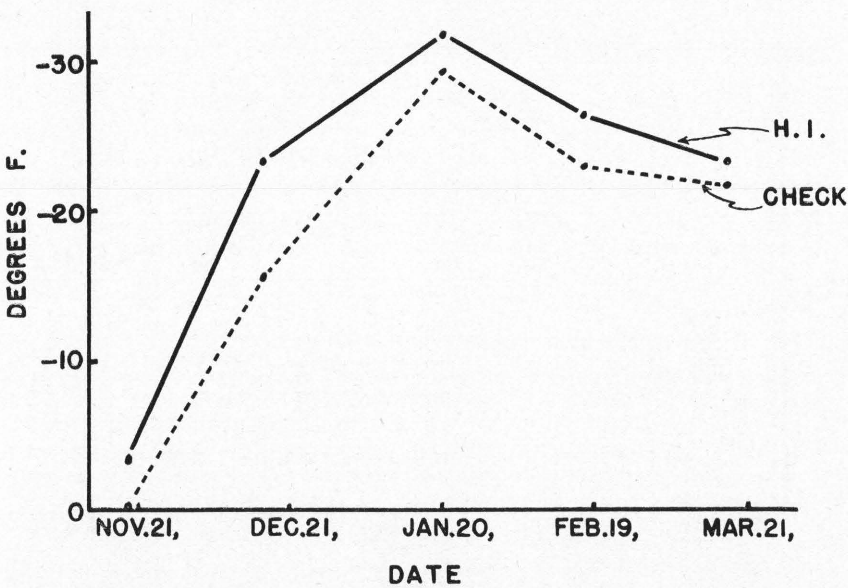


Figure 17. Effect of Hibernal interstock (H.I.) upon the cold hardiness of Staymared as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

Table XIV — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of three varieties of apples as influenced by various cultural treatments. Determinations were made at various dates during the winter.

Cultural Treatment	Temperature in Degrees F. Required to Result in 15 Percent Diffusion on					
	Nov. 5	Nov. 26	Dec. 18	Jan. 13	Feb. 19	Mar. 19
			Yellow Transparent			
Late N	-10.0	-21.0	-29.5	-29.0	-29.4	-21.0
Scoring	-14.1	-21.6	-37.8	-35.4	-32.0	-23.0
Defoliation	- 8.6	-19.4	-24.7	-22.7	-22.3	-20.6
Check	-17.3	-26.4	-33.5	-40.1	-34.5	-28.0
			Golden Delicious			
Late N	- 3.3	-12.0	-24.5	-29.5	-22.2	-18.3
Scoring	- 1.3	- 8.8	-17.5	-27.5	-21.8	-20.3
Defoliation	- 2.2	-18.8	-19.0	-27.0	-20.8	-20.5
Check	- 4.3	-11.8	-20.4	-29.5	-25.0	-17.0

From these data it is evident that the various cultural treatments exerted a definite influence upon the cold hardiness of the Yellow Transparent trees. On each of the various dates, those trees that had

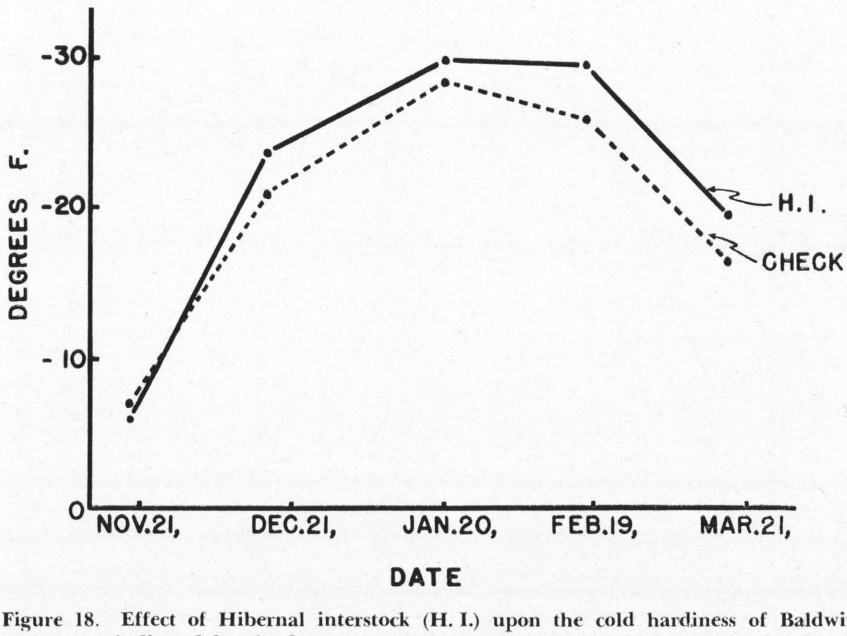
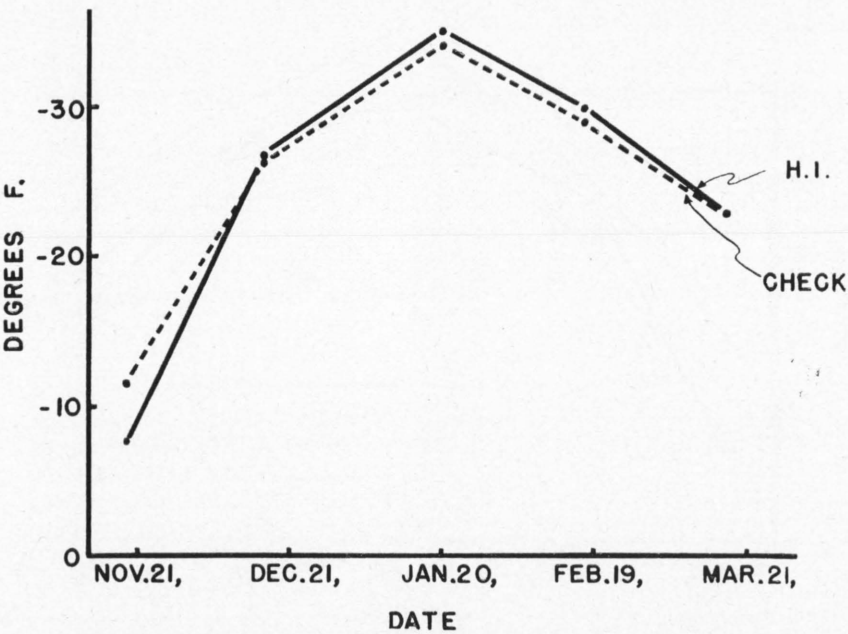


Figure 18. Effect of Hibernal interstock (H. I.) upon the cold hardiness of Baldwin as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

been subjected to the defoliation treatment were the most tender with the late nitrogen treated trees next. Terminal twigs from the check trees were more resistant to low temperature injury than were the scored trees on each of the test dates except December 18 (Figure 20).

From visual observations made during late summer and early fall it was noted that those Yellow Transparent trees that had been subjected to the defoliation treatment started to leaf out and bloom in late August. The new leaves that developed on these trees remained long after all leaves had fallen from adjacent check trees. It was also noted that the leaves failed to abscise from the late nitrogen treated trees until three to four days after they had fallen from the check trees.

Golden Delicious trees were subjected slightly later to the same treatments as the Yellow Transparent trees. The hardiness curves illustrating the influence of these treatments are given in Figure 21. The treatments conducted at the later dates had much less influence upon hardiness than did the same treatments carried out earlier in the season on Yellow Transparent trees. The data did show, however, that the defoliated trees were considerably more hardy on November 26 than



Figures 19. Effect of Hibernal interstock (H. I.) upon the cold hardiness of Delicious as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

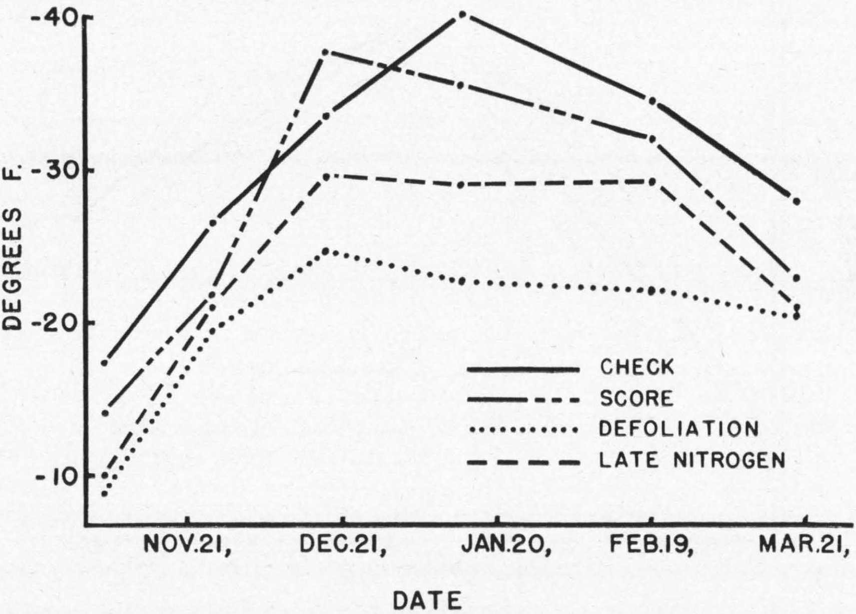
the trees subjected to any of the other treatments. The same was true of the late nitrogen treated trees on December 18.

From visual observations it was noted that after the scoring treatment was carried out on the Golden Delicious trees the leaves began to turn a bronze red color with the terminal leaves being the first affected. The Golden Delicious trees which were defoliated did not leaf out following treatment as did the Yellow Transparent trees. These Golden Delicious trees were somewhat lower in vigour, as indicated by less terminal growth, than the Yellow Transparent trees.

D. ROOT PRUNING STUDY

Hardiness determinations were made to study the influence of root pruning upon the cold hardiness of terminal twigs of Wolf River trees and are presented in Table XV and in Figure 22.

These data show that the root pruned tree was more resistant to low temperature injury than the check tree throughout most of the dormant season. The greatest increase over the check tree was during the early part of the season and it was not until March 29 that the check trees exhibited as much cold hardiness as the root pruned tree. During the fall it was observed that the leaves fell from the root pruned



Figures 20. Effects of various treatments upon the cold hardiness of Yellow Transparent as indicated by the low temperature treatments necessary to cause injury resulting in 15 percnt diffusion of electrolytes from terminal twigs at various dates.

tree about two weeks before they fell from the check tree. This effect was probably due to a more rapid attainment of maturity.

Table XV — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of root pruned and non root pruned Wolf River trees at various dates during the winter.

Cultural Treatment	Temperature in Degrees F. Required to Result in 15 Percent Diffusion on Nov. 5	Nov. 26	Dec. 18	Jan. 13	Feb. 19	Mar. 19
Root Pruned	-10.1	-25.0	-33.5	-31.8	-31.0	-17.0
Check	- 9.4	-12.5	-27.5	-29.3	-29.4	-18.3

E. PRUNING STUDY

To determine the influence of pruning upon low temperature resistance, two Yellow Transparent trees were heavily pruned, while two similar trees were left unpruned as checks. Hardiness determinations were made at various intervals after pruning according to the procedures outlined for the 1953-54 studies. The results of these determinations are presented in Table XVI and illustrated graphically in Figure 23.

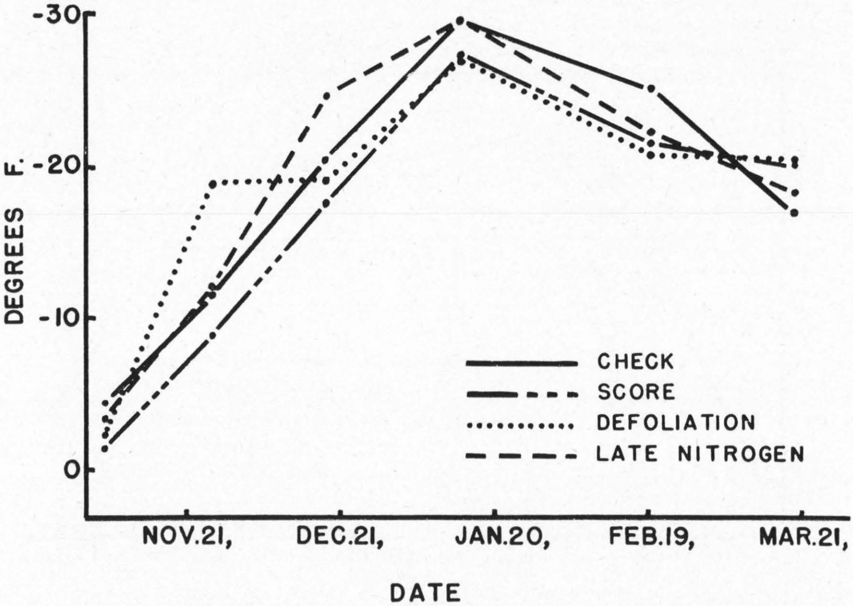


Figure 21. Effects of various treatments upon the cold hardiness of Golden Delicious as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

Table XVI — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of pruned and non-pruned Yellow Transparent trees at various intervals after pruning (Trees pruned on January 6, 1954).

Period after Pruning	Temperature in Degrees F. Required to Result in 15 Percent Diffusion when Pruned	Non-Pruned
7 hours	-36.5	-39.0
28 hours (1 day)	-32.5	-38.5
7 days	-36.0	-38.0
44 days	-35.0	-33.8

These data show that there was a definite reduction in the cold hardiness of Yellow Transparent terminal twigs directly after the tree had been pruned. This is illustrated by the fact that the low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes was 2.5°F. less for terminal twigs from pruned trees than for twigs from non-pruned trees 7 hours after pruning and 6.0°F. 28 hours after pruning. At the end of 7 days the difference between the two treatments was only 2.0°F. while after 44 days this was even less.

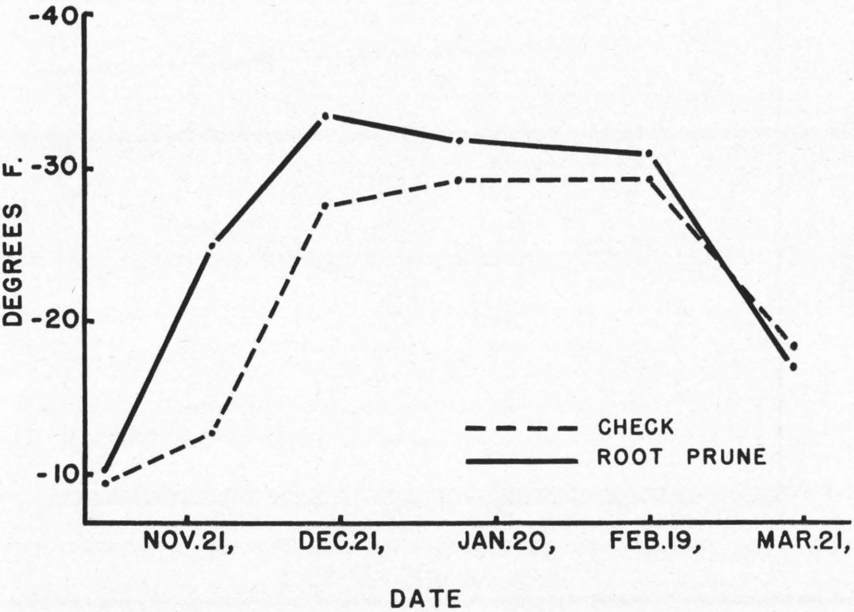


Figure 22. Effect of root pruning upon the cold hardiness of Wolf River as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

F. MALLING STOCK STUDY

Terminal twigs were collected from six different Malling stocks growing in stool beds. Hardiness determinations were made five times during the winter according to the procedures outlined for the 1953-54 studies. The low temperature values are presented in Table XVII and illustrated graphically in Figures 24 and 25.

Table XVII — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of various Malling stool bed materials collected at various dates.

	Temperature in Degrees Fahrenheit Required to Result in 15 Percent Diffusion on				
Variety	Nov. 26	Dec. 16	Jan. 13	Feb. 10	Mar. 19
Malling I	16.0	-27.0	-19.7	-19.0	-16.0
Malling II	- 0.0	-20.5	-27.8	-31.5	-19.0
Malling IV	-12.5	-30.0	-28.0	-22.5	-16.0
Malling V	-13.0	-27.0	-28.2	-29.0	-23.3
Malling VII	-13.3	-32.3	-24.2	-27.5	-15.5
Malling X	- 5.0	-23.0	-31.0	-27.0	-26.0

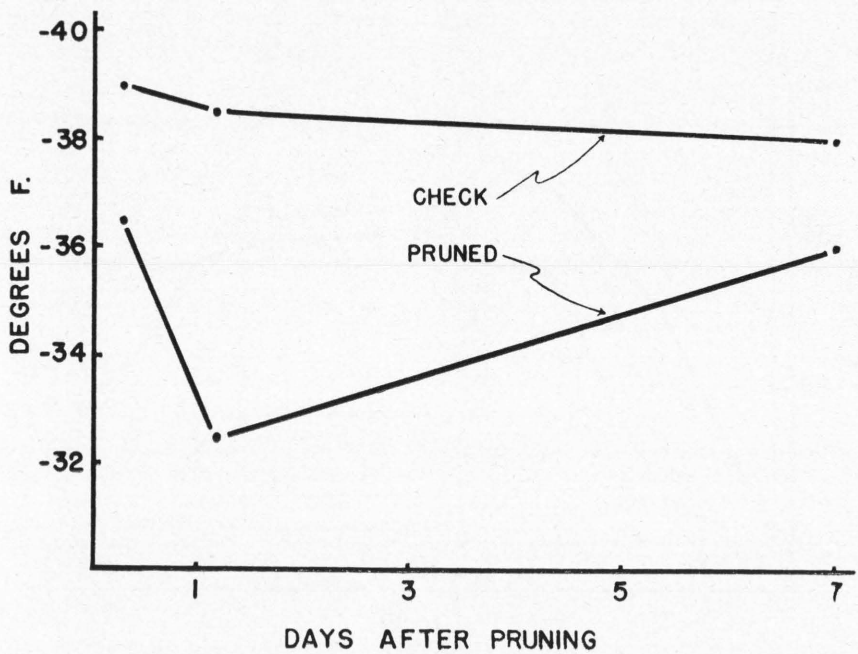


Figure 23. Effect of pruning Yellow Transparent trees on January 6, 1954 upon cold hardiness as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various intervals of time after pruning.

Terminal twigs from Malling II and Malling X were considerably more tender on November 26 than twigs from the other four Malling stocks. The data also show that in general those stocks which were more hardy early in the dormant season, November 26, attained maximum hardiness early and then decreased while those stocks which were more tender early in the dormant season did not obtain maximum hardiness until later in the winter. The one notable exception was Malling V which was relatively hardy early in the season and increased slightly in hardiness from December 16 to February 10, thus attaining maximum hardiness at this later date.

There was a considerable difference with respect to cold hardiness between the various Malling stocks tested at any one date. However, if the maximum hardiness for each of the stocks is taken irrespective of the date attained. the differences between individual stocks were small amounting to only 5.3 degrees fahrenheit. However, taking into consideration the entire dormant season, Malling V was the most hardy of the Malling stocks studied, while Malling I and II were the most tender. The fact that Malling II was decidedly more tender than the other root stocks early in the dormant season may well be an important

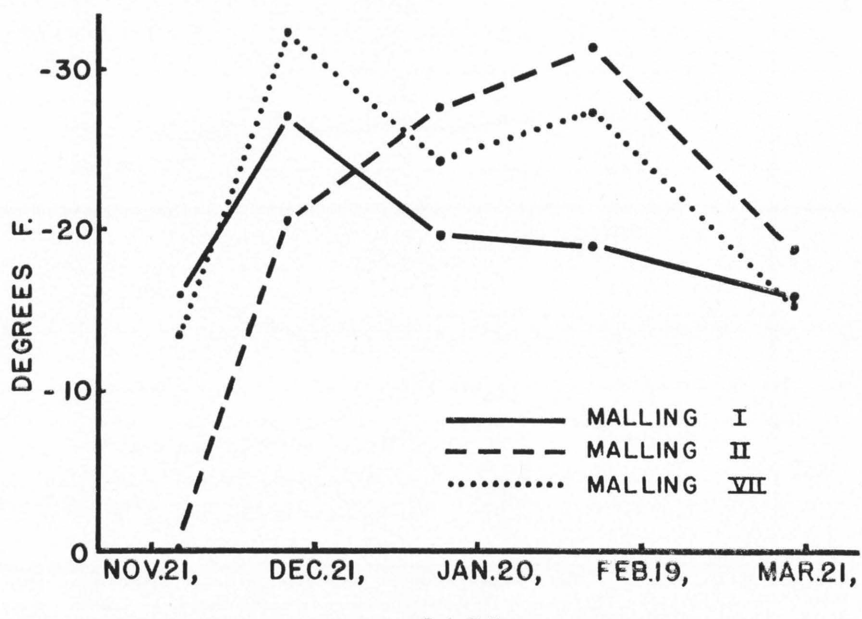


Figure 24. Variation between different Malling rootstocks with respect to cold hardiness as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates.

consideration of this study since the majority of the severe cold periods which have resulted in low temperature damage to apple trees since 1900 have occurred prior to December 15.

G. COLD HARDINESS AS INFLUENCED BY SEASONAL TEMPERATURE TRENDS

The cold hardiness of Golden Delicious twigs was determined on thirteen different dates throughout the fall and winter for the purpose of studying the influence of seasonal temperature fluctuations upon cold hardiness. These determinations were carried out according to the procedures outlined for the 1953-54 studies and the data are presented in Table XVIII. A detailed hardiness curve is presented in Figure 26 together with a graphic representation of the maximum and minimum daily temperatures recorded at the Ohio Agricultural Experiment Station during the fall and winter of 1953-54.

The data show that the hardiness of Golden Delicious trees increased steadily through November 12. However, a decrease in hardiness took place between November 12 and November 19 as indicated by the fact that the low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes was -11.6 degrees F. on

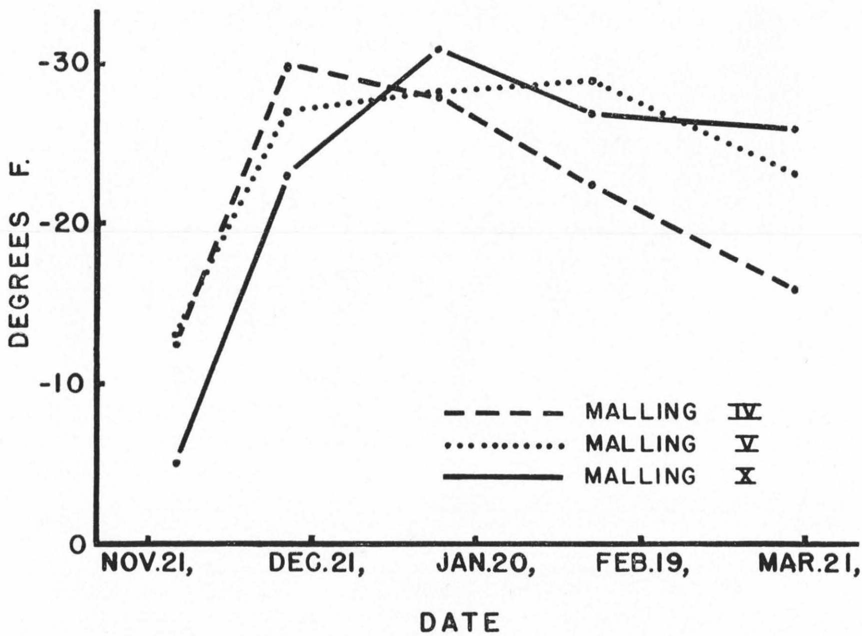


Figure 25. Variation between different Malling rootstocks with respect to cold hardiness as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates. (Graph 2).

Table XVIII — Low temperature treatment necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs of Golden Delicious trees determined at 13 different dates.

Date	Temperature in Degrees F. required to result in 15 percent diffusion
Oct. 18	+ 7.5
Oct. 25	+ 4.2
Nov. 5	- 4.3
Nov. 12	-11.6
Nov. 19	- 6.7
Nov. 26	-11.8
Dec. 9	-21.5
Dec. 16	-20.4
Jan. 6	-25.7
Jan. 13	-29.5
Feb. 10	-24.8
Feb. 19	-25.0
Mar. 19	-17.0

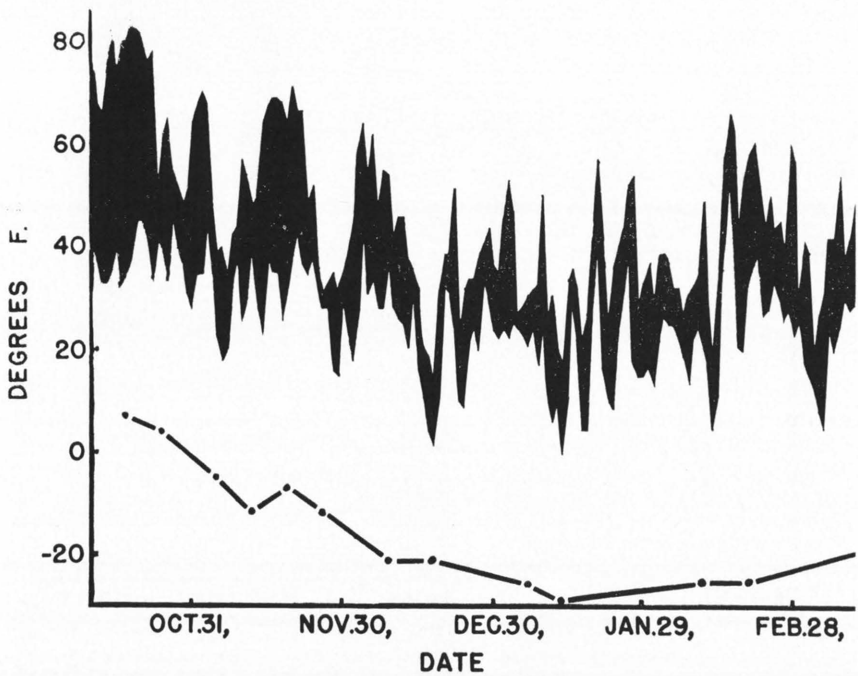


Figure 26. Cold hardiness of Golden Delicious, as indicated by the low temperature treatments necessary to cause injury resulting in 15 percent diffusion of electrolytes from terminal twigs at various dates from October 18 to March 19, in comparison with maximum and minimum daily temperatures.

the 12th, but only -6.7 degrees F. just one week later. Figure 26 shows that the temperatures during this same week were unseasonably high.

After November 19, twigs from the Golden Delicious trees increased steadily in hardiness to a maximum which occurred on January 13. It is interesting to note that this was also the precise day on which the lowest minimum temperature for the year was recorded. Following the January 13 peak the hardiness of Golden Delicious trees decreased steadily through March 19 which was the last date that cold hardiness determinations were made.

DISCUSSION

I. METHODS EMPLOYED IN COLD HARDINESS STUDIES

While many methods have been used to study the resistance of the apple to low temperature injury, the one most commonly employed in recent years is the Electrolytic Technique with various modifications. This technique as used by Swingle (56), Stuart (52), (53), (54), (55), Hilborn and Waring (33), Way (60), and Edgerton (25) has contributed greatly to our knowledge in this field. These same general techniques were used in the investigations reported herein. However, during this study modifications were made not only in procedure but also in the methods of hardiness comparisons in an attempt to expand still further knowledge in this area.

A. COLD HARDINESS COMPARISONS

Through the years cold hardiness evaluations of the apple have been generally made on the basis of the amount of injury caused by a specific low temperature treatment. This means of evaluation is satsisfactory providing the main objective of the evaluation is to compare the relative hardiness of a number of individuals on a specific date. It was this method of comparison that was successfully used in the 1950-51 studies to compare the relative hardiness of 55 varieties on four different dates. The principal objection to this means of hardiness evaluation is that it does not allow for an accurate comparison of the hardiness of an individual on two or more different test dates, unless identical low temperature treatments are used. It was for this reason that a number of experiments were conducted for the purpose of making basic changes in the methods of hardiness evaluation prior to the 1953-54 studies.

In modifying the methods for the 1953-54 work in order that the hardiness of an individual could be more accurately followed throughout the dormant season it was considered impractical to attempt to evaluate hardiness on the basis of injury inflicted by a specific low temperature treatment. It was realized that a low temperature treatment severe enough to cause detectable injury to all varieties in January would be far too severe a treatment if employed in November while a low temperature treatment suitable for November would be insufficient to cause detectable injury to many varieties in January.

Another disadvantage of any method where comparisons are based upon the injury resulting from a specific low temperature treatment is that the treatment must be severe enough to result in at least some injury to even the most hardy varieties tested and such a treatment would frequently cause very severe injury to some of the more tender varieties included in the test. If two or more of these less hardy varieties were to be compared it would necessarily be on the basis of injury which may be far in excess of that where recovery could take place.

Because of the above complications a method was developed by which hardiness comparisons could be made on the basis of the low temperature treatment necessary to cause a specific amount of injury. The method would essentially consist of subjecting five similar samples to five different low temperature treatments. The injury resulting from each of the low temperature treatments would be evaluated by means of the electrolytic technique. A curve could then be developed depicting the amount of injury in relation to these specific low temperature treatments. From such a curve any degree of injury expressed in terms of percent diffusion of electrolytes could be selected and the low temperature treatment necessary to cause the amount of injury easily determined. In this way comparisons could be made on the basis of the low temperature treatments necessary to result in a pre-designated amount of injury.

In selecting the amount of injury upon which to base comparisons two important considerations were taken into account. First, if possible the amount of injury should have at least some practical significance. The results of the experiment in which the percent diffusion of electrolytes and extent of tissue browning were compared indicated that the amount of injury resulting in 15 percent diffusion of electrolytes corresponded closely with the injury characterized by death of the cambium. Once the cells of the cambium layer have been killed no further growth can take place and thus it would be of little value to base comparisons on injury more severe than that which would result in death of the cambium. The decision was thus made not to base comparisons on injury resulting in more than 15 percent diffusion of electrolytes.

A second important consideration in selecting the degree of injury upon which to base comparisons was that it had to be such as to be identified accurately on a curve depicting percent diffusion of electrolytes in relation to the low temperature treatment. Because of the difficulty involved in the determinations, it was considered inadvisable to base comparisons on very slight degrees of injury, or those resulting in from 0 to 10 percent diffusion of electrolytes. Furthermore, since low temperature injury first took place in the innermost tissues, it is quite probable that such injury would have had little detrimental effect upon subsequent growth and development of the tree. It was on the basis of the above considerations that the injury corresponding to 15 percent diffusion of electrolytes was selected as the most suitable upon which to base comparisons.

In selecting that amount of injury corresponding to 15 percent diffusion of electrolytes, it was not intended to affix undue importance to this particular degree of injury and likewise it should not be taken to mean that a greater amount of injury would necessarily result in death of the particular tissue and a lesser degree in complete recovery. However, this value did afford a definite extent of injury upon which to base comparisons and it did represent a degree of injury generally characterized by death of the cambium.

B. ELECTROLYTIC TECHNIQUE REFINEMENTS

The success of the method of cold hardiness comparisons outlined for the 1953-54 studies was dependent upon the accuracy of the electrolytic technique in evaluating injury caused by low temperature treatments. For this reason a number of tests were performed for the purpose of refining the sensitivity of the technique in order to make the most efficient and effective use of available test material in studying cold hardiness.

In previous studies using the electrolytic technique maximum injury to tissues was caused by boiling. However, since low temperature injury is that which is being evaluated, it was considered advisable to determine if there was any significant difference between the diffusion of electrolytes from tissues severely injured by boiling and by low temperatures of -100°F. As a result two experiments were performed: one according to the general methods employed in the 1950-51 work and the other according to the 1953-54 methods. The results as presented in Tables VII and VIII show that there was no significant difference between the two means of inducing maximum injury as far as the diffusion of eelctrolytes was concerned. For this reason boiling, being the more convenient, was used throughout.

In order to utilize the procedures outlined for the 1953-54 studies it was essential that maximum utilization be made of the available test material. The experiment conducted to determine the most desirable segment length during the diffusion period showed that there was a considerably greater diffusion of electrolytes from samples composed of short segments than of long segments. The most probable explanation of this difference is that the diffusion took place almost entirely through the ends of the tissue segments and not through the bark. Likewise this diffusion occurred only from those tissues near the ends of the individual segments. The data indicate that although the diffusion from the terminal 1/4 inch of tissue was nearly complete, diffusion also took place from tissues further than 1/4 inch from the extremities, but at a reduced rate. Since the Electrolytic Technique is dependent upon the diffusion of electrolytes from injured tissues the centermost portions of longer segments would be of less value in this type of injury evaluation.

The data also indicated that the shorter the segment length, the greater the total diffusion of electrolyes taking place from a specific amount of that tissue. It should be kept in mind however, that total diffusion included electrolytes diffusing as a result of cut surface injury as well as internal injury. In studying the injury induced by a particular treatment the internal injury was the important consideration and thus the diffusion which resulted from the cut surface must be substracted from the total. The smaller the individual segments, the greater the number of these segments per sample and likewise, the greater the total cut surface. The increase in the cut surface as the segments were cut into the shorter lengths resulted in a considerable increase in the cut surface diffusion.

Taking both cut surface and internal injury into consideration the ½ inch segment (45 segments per sample) was considered to constitute the most desirable segment length to use. Segments of this length were short enough to allow for near maximum diffusion resulting from internal injury, yet not short enough to cause excessive diffusion to take place as a result of cut surface injury.

Once the length of the individual segments had been determined it became necessary to establish the most suitable size of sample. Figure 8 illustrates the effect of sample size upon total diffusion and thus specific conductance. Although specific conductance increased with increases in sample size, such increase was not directly proportional. Probably the rate as well as the total diffusion which occurred was dependent upon the difference in concentration of the external media as compared to the concentration of the internal solution.

In selecting the most suitable size of sample, two considerations were taken into account. The first was that the procedure, as outlined for the 1953-54 studies, involved the use of a large number of samples. Therefore, the smaller the individual sample which could be utilized without sacrificing accuracy, the more efficient the available test material for studying apple hardiness. The other consideration involved the sensitivity of the Solu-Bridge specific conductance determining device. This device was most sensitive for specific conductance values between 10 and 100 x 10^{-5} and for this reason it was considered advisable to select a sample size which would yield conductance values within this range. On the basis of the above considerations as well as the results of the experiments showing the influence of sample size on diffusion of electrolytes, 7 grams was selected as the sample size to employ in the 1953-54 work.

In the 1950-51 studies, 18 hours was selected as a diffusion period which was also the period used by Stuart (52). However, Swingle (56) stated that he made conductivity determinations whenever convenient after from 12 - 36 hours. In order to establish the most desirable diffusion period to employ during the course of the 1953-54 work the effect of diffusion period upon the diffusion of electrolytes was studied. It will be recalled that the results derived from this study indicated that there was a very rapid rate of diffusion at the very outset of the diffusion period, but that this rate decreased markedly after 15 - 20 hours. On the basis of the curve (figure 9) illustrating the influence of time on diffusion of electrolytes and because of convenience, 24 hours was selected as the diffusion period to be used throughout the 1953-54 work. A period longer than 24 hours was considered inadvisable due to the fact that fermentation, which affected the specific conductance, frequently took place soon after 24 hours. Also, at the end of 24 hours the rate of diffusion had decreased to the point where $\pm$15 or 20 minutes did not result in a detectable difference in the specific conductance determination. A diffusion period of less than 24 hours was not selected because of the convenience of the 24-hour period and because at least some additional diffusion of electrolytes occurred during the last 6 hours of the 24-hour diffusion period.

With respect to the preceding discussion it should be kept in mind that the success of the electrolytic technique is not dependent upon the complete diffusion of electrolytes from the test materials. The important consideration is that the technique be standardized in order that the results obtained be an accurate criterion as to the extent of apple twig tissue injury. It is for this reason that a 24-hour diffusion period was selected even though there may occur additional diffusion of electrolytes thereafter and that ½ inch segments may be used even though slightly more electrolytes might be removed from somewhat shorter segments.

C. ARTIFICIAL LOW TEMPERATURE TREATMENTS

In the 1950-51 experiments the tissues were held at the minimum temperature for a period of 6 hours. Stuart (52) on the other hand employed a 16-hour minimum temperature period. In these studies, since only one minimum temperature was used the longer period at the minimum temperatures could be employed. When the procedures were modified in 1953-54, instead of using one minimum temperature for each artificial freezing test, five were utilized. A two-hour period at each of the five minimum temperatures was considered to be the maximum practical period. However, before this two-hour period was chosen the influence of time at the minimum temperature upon the extent of injury was given consideration. The results of this study presented in Table IX and illustrated graphically in Figure 10 indicate that there was an increase in injury with increasing time at the minimum temperature and that this increased injury was most rapid during the first few hours. Both Potter (46) and Hildreth (34) found that increased injury resulted from longer periods at the minimum temperature.

On the basis of the curve depicted in Figure 10 two hours was established as a suitable exposure time interval. The important consideration with regard to time at the minimum temperature is that uniformity be maintained and that all samples be subjected to the same period of time at the minimum temperature if valid comparisons are to be made.

II. THE INFLUENCE OF VARIOUS FACTORS UPON COLD HARDINESS

The remainder of this discussion will be devoted to the various factors which effect the hardiness of the apple, as evaluated by the methods previously described.

One of the most important factors concerned with cold hardiness is varietal variation. Horticultural literature is filled with examples of variation between varieties with respect to cold hardiness. Numerous evaluations have been made following so called test winters. On the basis of these test winter evaluations workers have in general concluded that Baldwin, Stayman Winesap, and Rome Beauty were more tender than Northern Spy, Golden Delicious, and Jonathan. In these same evaluations the varieties McIntosh, Yellow Transparent, Hibernal, and Virginia Crab were generally classified as possessing a high degree of resistance to low temperature injury.

In the 1950-51 work 55 varieties were evaluated on the basis of relative hardiness at four different times during the dormant season. The results of the fall test agreed closely with these contemporary opinions on the relative hardiness of apple varieties. No such agreement was found to exist, however, between the test winter evaluations and the hardiness determinations carried on later in the dormant season. A possible explanation of this variation may be found from a study of past reports upon which such opinions were presumably based. As indicated in the literature review in all but two of the test winters since 1900 the low temperature injury to apple trees occurred during the early part of the dormant season (prior to December 15). The two exceptions are the winters of 1917-1918 and 1935-1936 and in each of these cases workers reported that fall frosts adversely affected the normal maturing processes of the trees. Thus, it appears that contemporary hardiness concepts are based on injury which occurred primarily at that time of the year designated as fall in the 1950-51 studies and that these concepts reflect the hardiness of varieties for that portion of the dormant season only.

The data assembled during the course of the 1950-51 studies strongly indicated that the relative hardiness position of a variety is not a constant value but that it generally undergoes significant changes with time. (See Figure 13) On the basis of these results speculations were made that some varieties may mature, or harden, more slowly in the fall and as a result are more tender than other varieties that may have matured more rapidly. However, the initially tender variety given time to mature fully may be the more hardy variety later on. Also, on the basis of these test results it may be speculated that some varieties may lose their hardiness qualities more slowly than others towards the end of the dormant season and thus be relatively hardy in early spring.

The above speculations stimulated considerable interest in the "hardiness curves" of individual varieties and was primarily responsible for the initiation of the 1953-54 studies utilizing methods that would yield data to provide answers to some of the problems that arose during the 1950-51 work.

In the 1953-54 studies hardiness curves for nine different varieties were developed and are presented in Figures 15 and 16. The results of these studies through January 20 conformed very well with the data obtained during 1950-51 indicating that varieties Staymared, Baldwin, and Rome Beauty were the most tender and Delicious somewhat more hardy. The fact that the varieties Hibernal and Virginia Crab were found to possess a high degree of cold hardiness likewise coincided with past evaluations. Malus Robusta #5, included in this investigation because it is currently recommended in Canada as a hardy stock, and Columbia, which has also been referred to as a possible hardy stock, were found to be very hardy. It is interesting to note that the variety Franklin which is the result of a McIntosh x Delicious cross is more hardy than Delicious which would indicate that at least some of the hardiness characteristics of McIntosh were inherited by Franklin. The hardiness determinations made on February 17 and March 17 did not conform to the experience of test winter evaluations referred to previously, thus confirming the results obtained in the 1950-51 studies.

The hardiness curves in Figures 15 and 16 also illustrate the fact that the relative hardiness of a variety at any one date was only a part of the overall hardiness characteristics of that variety. Varietal differences likewise existed with respect to maximum hardiness, rate of hardiness increase, date of maximum hardiness as well as rate of hardiness decrease. It should also be noted that varieties more hardy early in the dormant season attained the greater maximum hardiness and likewise attained it earlier than did some of the more tender varieties. The marked differences between the hardiness curves of the various varieties is undoubtedly due to genetic differences which influence the various factors which are associated with tissue maturity, or "hardening off," and thus cold hardiness.

The influence of the genetic constitution of the trees is by no means the only factor affecting the hardiness curve but is undoubtedly one of the most important. Daily temperature conditions likewise exert a decided influence upon the expression of the hardiness of any particular tree but the genetic make up of the tree is still more important. This is evidenced by the fact that some of the more hardy varieties such as Columbia and Malus Robusta #5 which attained maximum hardiness early, and likewise started to lose hardiness early, were decreasing in hardiness while during the same period of time some of the more tender varieties such as Baldwin and Staymared were still increasing in hardiness under the same daily temperature conditions. Another example of the decided influence of genetic factors upon the hardiness curve is afforded by the fact that Malus Robusta #5 was second only to Columbia in hardiness on December 16, but yet was the most tender variety studied on March 17.

The hardiness curves which were developed for the six different Malling stocks (Figures 24 and 25) likewise illustrate the varietal differences which existed with respect to hardiness. Especially noticeable was the fact that Malling I, IV, V, and VII, which were the more hardy stocks early in the season, attained maximum hardiness nearly two months earlier than did Malling II which was by far the most tender early in the dormant season. Possibly the most important characteristic of a variety with respect to cold resistance is its hardiness early in the dormant season and the rate at which that variety attains maximum hardiness. As was pointed out earlier, the major part of the low temperature injury which has been suffered by apple trees since 1900 has ocurred early in the season and thus those varieties more tender at this time will be those most severely injured.

A Hibernal interstock piece evidently imparted to the scion variety grafted upon it a slight increase in cold resistance. However, in the evaluation of this effect other factors, notably tree vigour, must be taken into consideration. If the vigour of the tree is increased as a result of a Hibernal interstock piece, this increased vigour would result in a reduction in hardiness, especially early in the dormant season. This is because a vigorous tree will continue active growth longer resulting in a delay in the normal maturing processes. Thus the increased hardiness effect of the Hibernal interstock may be modified or even eliminated if this interstock results in a more vigorous tree. The above discussion is more clearly understood after examination of Figures 17, 18, and 19. There was no visible difference in the vigor of the Staymared trees on Hibernal interstocks and on seedling roots, however, the Staymared trees on Hibernal interstocks were more hardy (Figures 17). In the case of the variety Baldwin the Hibernal interstock trees were slightly more vigorous and as a result the reduced hardiness due to the added vigor offset the increase in hardiness due to the Hibernal interstock on November 19, however, the interstock trees were the more hardy during the remainder of the season (Figure 18). With the variety Delicious the Hibernal interstock trees were more vigorous and this increased vigor more than offset the increased hardiness effect of the Hibernal interstock early in the season, however, the two effects balanced each other on subsequent test dates (Figure 19.) It should be pointed out that ordinarily trees on Hibernal interstock are smaller, make a reduced rate of growth and the maturity of the scion wood is attained earlier, however, none of these effects were evident in the case of the varieties included in this study.

The increased hardiness of the scion variety caused by a Hibernal interstock generally confirm the observations of Edgecombe (24) who noted that after the 1940 freeze there was less injury to twigs of Jonathan, Turley, and Stayman Winesap when these varieties were worked on Hibernal than when grown on seedling roots. These data also confirm the results of Hilborn and Waring (33) who have shown that Hibernal interstocks have resulted in an increase in the hardiness of scion varieties. However, they found that Virginia Crab was even more effective in inducing this effect. Stuart (52) found that the hardiness of the scion variety was not influenced by the stock, however he worked with one year budded nursery stock and Filewicz (26) in Poland stated that the tender scion took on the hardiness of the stock within 5 years. It is possible that Stuart would have attained results similar to those of Filewicz had his trees been somewhat older and better established.

Aside from varietal differences, lack of maturity has been more frequently mentioned as a contributary cause of low temperature injury than any other one factor. This is best characterized by an observation made by Anthony et al. (2) after surveying the injury to apple trees following the severe cold period which occurred on January 22, 1936.

These authors reported that at first there seemed to be much conflicting evidence until it was realized, as had been reported in connection with previous test winters, that maturity was, once again, the common factor throughout.

After careful study of the effects of the various cultural treatments upon the cold hardiness of the Yellow Transparent trees it was noted that reduced rate of attainment of maturity was possibly the important factor here also (Figure 20). The greatest reduction in cold resistance resulted in those trees which had been subjected to the defoliation treatment. These trees were defoliated before the buds had become dormant and as a result new leaves formed. This flush of active growth which took place late in the summer resulted in a delay in the normal maturing processes of the tree and undoubtedly was the cause of the marked reduction in cold hardiness. A similar, yet somewhat less pronounced, effect upon cold resistance of Yellow Transparent trees resulted in the case of the late nitrogen treated trees. The effect of this treatment may likewise be attributed to a prolongation of the active growing season and thus a delay in the normal maturing processes of the tree.

The scoring treatment also caused a general reduction in the hardiness of Yellow Transparent trees but the effects were not as pronouned as they were in the cases of the defoliation and late nitrogen treatments. The reason for the difference in results obtained is not clear. However, it is entirely conceivable that the scoring treatment had the same general effect, yet to a lesser degree, as the defoliation and late nitrogen treatments, in causing a slight retardation in the normal maturing processes of the tree. The scoring treatment carried out on August 21 possibly resulted in a reduction in the downward translocation of the products of photosynthesis and might not affect, to any degree, the upward movement of solutes. By causing the products of photosynthesis to remain in the upper portions of the tree at this time, late August, it is possible that active growth was slightly prolonged thus retarding the normal maturing processes of the tree, which in turn would result in a reduction in hardiness. Such an effect of scoring however would be slight.

Factors associated with a delay in maturity resulted in a reduction in cold hardiness not only during the early part of the dormant season but throughout the remainder of the dormant season as well, with the maximum differences in cold resistance occurring on January 23. In addition to causing a reduction in maximum hardiness, or cold resistance attained by the particular tree or trees in question, the Yellow Transparent trees subjected to all three treatments attained maximum hardiness at an earlier date than the check trees. (Figures 20.) Both Chandler (14), in discussing the injury resulting from a freeze occurring on December 20, 1917, and Anthony et al. (2) in discussing the effects of a January 22, 1936 freeze mention that lack of maturity was an important factor. The observations of those workers would then also indicate that those conditions which result in a delay in maturity likewise exert an influence upon cold hardiness later in the dormant season as well.

Golden Delicious trees were subjected to the same three treatments, defoliation, late nitrogen and scoring as had been the Yellow Transparent trees above mentioned. The effects of these treatments with respect to the cold resistance of Golden Delicious are graphically presented in Figure 21. In the evaluating these results two important considerations should be kept in mind. First, the Golden Delicious trees were subjected to the treatments in question around September 1, or 2 to 3 weeks later than were the previously mentioned Yellow Transparent trees. Secondly, the Golden Delicious trees were in a somewhat lower state of vigour than the Yellow Transparent trees as was indicated by shorter terminal growth.

Thus, the effects of the three treatments in question upon the cold hardiness of the Yellow Transparent trees was to delay the normal maturing processes of the tree through a delay in the cessation of active growth. However, in the case of the Golden Delicious trees the effects of these same three treatments upon cold resistance were only slight, of a different nature, and possibly not generally significant. The treatments evidently had no effect upon the normal maturing processes of the tree which was quite likely due to the later dates on which they were treated. They did however, exert some influence upon the cold resistance of the trees. The defoliation treatment trees were more hardy than the trees of the other treatments on Novmber 26 and the same was true of the late nitrogen treated trees on December 18. Over this same period the scored trees were slightly more tender than the rest. A possible explanation of the effects of the various treatments upon the Golden Delicious trees may only be postulated. Anthony et al. stated (2) that trees in a normal state of vigour are more resistant to low temperature injury than similar trees either in a higher or lower state of vigour. If this be true, the effect is possibly associated with the presence and concentrations of the various cell constitutents which in the case of a tree in a normal state of vigour are such as to develop the highest degree of cold resistance possible within the limits of the genetic constitution of the tree. However, if the tree is below normal in vigour the conditions within the cells would undoubtedly be somewhat different and if this situation could be adjusted to more nearly correspond to that present within the cells of a tree of normal vigour the cold hardiness of this tree might possibly be increased.

In line with the above thinking it is interesting to note that in the case of the Golden Delicious trees those treatments which would be expected to result in proportionately greater concentrations of the various nitrogen fractions in the above ground portions of the tree, or in other words the defoliation and late nitrogen treatments, resulted in the increased hardiness of the trees subjected to these treatments. The defoliated trees were more hardy than the rest on November 26 and the same was true of the late nitrogen trees on December 18 which was as might be expected since the changes in the cell constitutents likely occurred earlier in the case of the defoliation treatment trees. The scoring treatment which would have the direct opposite effect, that being to cause an increase in the carbohydrate fractions, resulted in a slight but never the less decreased hardiness during the early part of the dormant season.

In addition to those treatments discussed above, the effect of root pruning upon cold hardiness was also studied. (Figure 22). It is evident that this treatment resulted in an increase in hardiness throughout the greater part of the dormant season, being especially pronounced on November 26. The latter part of the 1953 growing season in Wooster was very dry and thus the severe root pruning accentuated the effect of drought. The reduced water uptake resulted in a premature hardening which was exemplified by the fact that the leaves fell two weeks earlier from the root pruned tree than from the check tree. This reduced water uptake presumably resulted in a reduced amount of free water in the tissues which in turn was quite likely the important factor concerned with the increased hardiness of the root pruned tree early in the dormant season. Later in the season the free water possibly increased to a level more near that of the adjacent check tree and thus the difference between the two trees with respect to cold resistance was considerably reduced.

Several workers (11) (9) (2) (32) have cited examples of instances where trees pruned prior to a cold period have been more severely injured than those trees not so pruned. These observations are substantiated by the results of this study. (Figure 23). The reason for the sudden and decided loss of hardiness within 28 hours after pruning can only be postulated. Such a rapid action may have come about due to an increase in the free water fraction of the remaining tissues because of a reduction in tree volume. This would be the effect if water absorption took place for a short time after pruning at the same rate as before pruning. Possibly by the end of a week the rate of water absorption had decreased proportionately to the reduced amount of above ground tissues a condition which would permit equilibrium to be once again established between the various water fractions of the tissues thus resulting in reestablishment of the original cold hardiness.

Daily temperature conditions played a very important role in tree hardiness as illustrated by the detailed hardiness curve for the variety Golden Delicious (Figure 26). The cold resistance of an apple tree was found to increase as the daily temperature dropped. Likewise, if there occurred a period of unseasonably warm weather an appreciable reduction in cold hardiness resulted. This fact is illustrated by the reduction of hardiness of Golden Delicious due to the unseasonably warm weather that took place between November 10 and 20. The results obtained also offer a clue as to the reason for the rapid drop in temperature following a warm period resulting in more severe injury than a more moderate rate of temperature drop. The warm weather would result in a reduction in cold hardiness leaving the tissues more susceptible to low temperature injury. However, if the temperature drop be moderate the hardiness of the tissues would increase with the temperature drop and thus the injury resulting from a particularly low temperature attained shortly thereafter would be less severe than would have been the case had the temperature drop been more rapid.

While temperature conditions do have marked effects upon cold hardiness, other factors are fully as/or even more important. For example, as has been illustrated in this investigation, both genetic as well as environmental factors (excluding temperature) exert an influence upon rate of hardiness increase, maximum hardiness, date maximum hardiness is attained, and rate of hardiness loss. All of these effects however, are also dependent upon low temperature for expression. In other words, no one factor is all important in the development of cold resistance within an apple tree. Absolute cold hardiness at any one date is the result of the influence of many factors.

III. PRACTICAL IMPLICATIONS OF RESULTS OBTAINED

Low temperature injury has caused the death or crippling of thousands of apple trees. This injury occurs with distressing regularity and is a more common problem than is generally recognized. It is not restricted to the colder climates but is also a serious problem in southern apple producing areas. A considerable amount of the injury that has occurred in the past could have been avoided if the cold hardiness of the apple had been more completely understood.

A portion of the work reported in this bulletin was devoted to the development of a technique which would yield information necessary for a more complete understanding of cold resistance in the apple. The method developed and described made it possible to follow the hardiness of an individual variety through the entire dormant season and to consequently study the influence that various factors might exert upon its hardiness curve.

One of the more important facts revealed from an evaluation of the hardiness curves was that once past the early dormant period, the apple attains sufficient hardiness to withstand rather severe low temperature periods and that the relative comparison of varieties or treatments at this time would have little commercial significance. The most vulnerable period is early in the dormant season and those varieties that are slow to attain cold resistance in the fall and those treatments that delay the normal development of hardiness are the most likely to be associated with low temperature injury in the orchard.

Low temperature injury is usually thought of as a cold climate problem. This is not necessarily true. The early fall is the most vulnerable period and serious damage may be caused by temperatures not normally considered severe provided the tree has not yet attained a significant degree of cold resistance. Such conditions are as likely to occur in the South as in the North. Low temperature injury has been frequently observed in the orchards of Virginia and North Carolina.

In recent years there has been an increasing tendency for growers to encourage the rapid vegetative growth of non-bearing trees in an attempt to develop as large a bearing surface as possible by the time the trees come into production. Such practices result in young trees that are very vigorous and frequently delayed in their normal hardening.

Another practice that may still further aggravate the problem in the future is the wider use of milder pesticide spray chemicals. In the past apple growers were forced to use spray chemicals, such as lime-sulphur and Bordeaux which were phytotoxic. This resulted in earlier leaf drop and encouraged earlier hardening of the trees. The currently used, less phytotoxic spray chemicals allow the foliage to remain in a more succulent condition longer and consequently delay the normal hardening of the trees. This should result in a reduction in cold resistance during the most vulnerable period of the year.

The hardiness curves for a number of different varieties illustrate that some varieties, particularly, Staymared, Baldwin, and Rome Beauty are slow to attain cold resistance in the fall and as a result growers must be particularly careful with these varieties and not carry on those practices which will further delay the normal maturing process (Figures 15 and 16).

The results of the cultural treatment study indicate that any practice which will stimulate a high level of vigor and/or retard normal hardening will increase the potential hazard of low temperature injury. These results have greater commercial importance now than ever before because the increased use of milder spray programs may already have increased the potential hazard. It is also significant that competition in the fruit industry has never been more intense and vacant spaces in an otherwise good planting may quickly convert a profitable orchard to a profitless operation.

The results of the pruning study show that for a short period after a tree is heavily pruned, the cold resistance of that tree is reduced. Growers pruning prior to the first of the year should avoid pruning the more tender varieties if severe low temperatures are predicted within a few days.

In recent years there has been an increased interest in the use of size controlling rootstocks in commercial plantings. An important consideration in determining the most suitable stocks to use is the cold resistance of the stocks. This is an important factor whether the stock is used as a rootstock or as an intermediate stem piece. Malling VII and Malling II are two rootstocks that are currently receiving considerable attention for inclusion in commercial plantings. The implications of this study are that Malling VII would be more resistant to low temperature injury during the critical early dormant period than would Malling II. This factor should weigh heavily in the final selection of a rootstock for a commercial planting. If Malling II is to be used the grower should conscientiously avoid those practices which would delay normal tissue hardening.

The hardiness data clearly showed that Delicious and Stayman Winesap and their mutations were the least resistant to low temperature injury during the fall of any combination of varieties. Rome Beauty, Golden Delicious, Blackmack, Melrose, and Baldwin were also in the same category. Among the so-called hardy varieties of Canadian and Russian origin were Pippin Shafran, Noir de Vitry, and Kulon Kitaika. Among the varieties of similar origin which were very resistant to low temperature injury were Hibernal, Columbia, Anaros, Antonovka Shafran, Beauty Crab, Garnet Crab, and Virginia Crab. These however, are not equally satisfactory for use as intermediate stocks. Virginia Crab is now being discarded because of the hazard of stem pit virus when it is utilized as an intermediate stock. Hibernal has too often produced trees with the lower primary scaffolds insufficiently firm thus resulting in the branches bending to the ground. In an experiment at the Mahoning County Farm involving a considerable number of these varieties as intermediate stocks for Jonathan and Gallia Beauty only Columbia consistently gave the highest accumulated total yield per tree at the end of 12 years. Kulon Kitaika produced a very satisfactory yield but its hardiness appeared to be considerably less than that of Columbia. From these results it would seem that Columbia offers the most satisfactory possibility as an intermediate stock for Jonathan and Gallia Beauty. Whether it would be equally satisfactory for the red strains of Delicious is unknown but at least it should be considered for this purpose.

Likewise Columbia might well be utilized as has been suggested in Canada as a source of hardy seedlings to be used as rootstocks. However, the precise hardiness of the offspring would depend upon the resistance of the male parent to low temperature. For this purpose one of the hardy varieties might well be used as the pollen parent in order that outstanding hardiness will be retained in the seedlings.

While growers are powerless to modify many of the environmental factors that result in low temperature injury in the orchard there are certain precautions that can be taken to reduce potential hazards. Prior to planting the relative hardiness of varieties, rootstocks, interstocks, etc., should be given serious consideration in planning the orchard. Once the orchard is established care should be taken to avoid those cultural practices that will result in excessive vigor and delayed hardening. One of the more significant aspects of the work reported in this bulletin is that now a method is available which may be utilized to study other practical aspects of apple hardiness.

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